Myoadenylate deaminase deficiency

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A study of its clinical significance

Myoadenylate deaminase deficiency

PROEFSCHRIFT

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Ter herinnering aan mijn vader,
voor ma, Irene en Steef.

Contents

ABBREVIATIONS

ADA : adenosine deaminase
Ado : adenosine
ADP : adenosine diphosphate
AMP : adenosine monophosphate
AMPD : adenosine monophosphate deaminase
ANOVA: analysis of variance
ATP : adenosine triphosphate
CP : creatine phosphate
EC : energy charge
E/I : energy expenditure per unit of performance
GDP : guanosine diphosphate
GTP : guanosine triphosphate
Hx : hypoxanthine
IMP : inosine monophosphate
Ino : inosine
LA : lactate
MAD : myoadenylate deaminase
MVC : maximal voluntary contraction force
NAD^+ : nicotinamide adenine dinucleotide
NH_3 : ammonia
PNC : purine nucleotide cycle
S-AMP : succinyl adenosine monophosphate
TAN : total adenine nucleotides
TI : total impulse

The enzyme myoadenylate deaminase (MAD), or skeletal muscle adenosine monophosphate deaminase (AMP-deaminase, AMPD), underwent a revival of interest by an article of Fishbein[1] in 1978 with the intriguing title: 'Myoadenylate deaminase deficiency: A new disease of muscle'. The author describes the discovery of a new disease by screening muscle biopsies with a new histo-enzymatic stain for MAD. Although the deficiency of the enzyme as such was described before,[2] Fishbein associated it for the first time with the existence of myopathy in a causal relationship. Surprising is the suggestion that the deficiency state constitutes the most common enzyme deficiency of skeletal muscle![3] However, the complaints of this myopathy due to the absence of MAD are ill-defined and comprise exercise-induced easy fatigability, muscle-aches and -cramps. Although this exertional myalgia is a very common set of complaints in daily life and clinical practice, its causes are infrequently satisfactorily captured. Besides, the existence of myoadenylate deaminase deficiency as a disease sui generis was questioned soon after the first report.[4]

The high prevalence of the deficiency state and the possible causative relation with the commonly occurring complaints of exertional myalgia made it seem worthwhile to study the enzyme deficiency. In particular a screening test for the deficiency, evading the necessity of taking muscle biopsies, might be promising in resolving questions concerning the relation between the deficiency state and clinical complaints. Additionally, the study of the consequences of MAD-deficiency on the purine metabolism and the energy economy of skeletal muscle might shed some light on the hitherto unrewarding attempts at solving the problem of muscle fatigue.

In chapter I the aims of this thesis are outlined, preceded by a review of the literature relevant to the questions that have arisen. Their subsequent answers are formulated in six papers, which make up the chapters II to VII. The thesis is completed with a general discussion of the results obtained in the study (chapter VIII).

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Chapter I

INTRODUCTION AND OUTLINE OF INVESTIGATION

I.1. Clinical features in MAD-deficiency.

The main clinical features reported in the first 5 patients with myoadenylate deaminase (MAD) deficiency as a disease state in itself, were rapid muscle fatigue, weakness and/or cramping following moderate to vigorous exercise,[1] which have since then been confirmed repeatedly by the same and other authors.[2,3,4] After the first description of these 5 deficient patients it became clear that this set of symptoms, commonly denoted as 'exertional myalgia', was not distinctive or characteristic: muscle weakness and fatigue can have a variety of causes ranging from disturbances in the central or peripheral nervous system to impaired function or destruction of the contractile machinery of skeletal muscle (Fig. 1).[5]

Figure 1. Chain of command for muscular contraction and the possible mechanisms underlying fatigue. (After R.H.T. Edwards, 1983)[5]

	Possible fatigue mechanism:
'Psyche'	
Brain	
↓	Impaired motivation i.e. neural motor
	← drive and motor unit recruitment
Spinal Cord	
↓	← Impaired reflex drive
Peripheral Nerve	
↓	← Impaired neuromuscular transmission
Muscle sarcolemma	
↓	← Impaired action potential
Transverse tubular system	← K^+, Na^+, H_2O imbalance
↓	← Impaired excitation
Ca^{++}	
↓	← Impaired activation
	← Impaired energy supply
Actin-myosin interaction	
↓	
Cross bridge tension + heat	← Thermal damage
↓	← Sarcomere damage
Force output	

As can be seen in Figure 1, the command chain for voluntary muscular activity thus involves many steps and force failure – that is fatigue – may be due to a breaking of anyone of the links of the chain of command.[6] When keeping in mind that ATP is the ultimate muscle energy source for contraction and the involvement of MAD in ATP-catabolism, one obviously starts looking for a possible relationship between muscle fatigue and a derangement, if any, in ATP metabolism in MAD-deficiency. A factor which hampers the description of the clinical picture is the frequent association of MAD-deficiency with other well-defined disorders, e.g. hypokalaemic periodic paralysis,[7] amyotrophic lateral sclerosis,[8] Kugelberg-Welander syndrome,[8] progressive systemic sclerosis,[9] dermatomyositis[2] and so on. So far, for instance, there have been described, irrespective of our own cases, at least 95 patients with MAD-deficiency, of which 50 were suffering from another disorder as well (see Table 1). So

there is no strongly suggestive constellation of signs and symptoms and most cases of the deficiency state are identified incidentally by routine application of the adenylate deaminase stain on muscle biopsies.[10]

Table 1 Associated disorders in MAD deficiency described in literature

	Number of MAD deficient patients	Associated disorders (number of patients in parentheses)
DiMauro et al [11]	1	none
Engel et al [7]	1	Primary hypokalaemic periodic paralysis (1)
Fishbein et al [12]	58	30 patients with a broad spectrum of other neuromuscular diseases
Gertler et al [9]	1	systemic sclerosis (1)
Hayes et al [13]	1	none
Heffner et al [14]	6	collagen vascular disease (3)
Joosten et al [15]	4	neuropathy (2)
Kar et al [3]	6	dermatomyositis (2) systemic sclerosis (1)
Kelemen et al [16]	1	none
Kelemen et al [3]	6	congenital myopathy (2) amyotrophic lateral sclerosis (1)
Mercelis et al [17]	1	facial and limb girdle myopathy
Rossi et al [18]	1	none
Scholte et al [19]	2	skeletal muscle type I atrophy and cardiomyopathy (2)
Shumate et al [8]	6	Kugelberg-Welander syndrome (1) amyotrophic lateral sclerosis (1) dystrophy (1)

If one takes into account the clinical diversity of the patients with an associated other illness, it is not surprising that several authors have questioned the existence of MAD-deficiency as a disease sui generis, suggesting that MAD-deficiency might not in itself produce symptoms, signs or complaints. The strongest advocates of this view are Shumate et al.[8,20] but also Heffner[14] and Hayes et al.[13] can be classed with them. Shumate[8] found on an initial screen of 256 biopsies 6 with absent enzyme activity. The clinical findings were so heterogeneous that they suggested MAD-deficiency was an incidental finding or produced symptoms only in concert with other, as yet unidentified, abnormalities or circumstances. The same argument was used by Heffner,[14] who found 6 deficient patients out of a series of 249 unselected, consecutive muscle biopsies, but among these an undetermined number of patients with an exertional myalgia. Hayes et al.[13] emphasized the need to look for an additional metabolic defect in patients with MAD-deficiency on the basis of their findings in two brothers who had virtually identical clinical features of an exertional myalgia whereas only the elder brother lacked the enzyme. Although Hayes et al. do not refer to the possibility of heterozygosity in the symptomatic yet not deficient brother, this issue is open to debate as is suggested by Fishbein et al.[21] We shall go into the matter of genetics later on. On the basis of the same arguments as brought forward by the preceding authors, Kelemen et al.[16] were inclined to be sceptical, but in the course of evaluating patients

with chronic exertional myalgia they biopsied 36 index patients, of which 3 (8.3%) lacked MAD. MAD-deficiency was much more common in patients with myalgia (8.3%) than in patients who underwent a muscle biopsy for other clinical problems (1.6%); hence their final remark that these findings seem to refute the view of Shumate et al.

Summarizing the data in this paragraph:

1. In MAD-deficiency there is no characteristic set of clinical signs or symptoms. There are two major situations in which MAD-deficiency is discovered: in cases of myalgia where in the absence of other abnormalities the MAD-deficiency probably plays a causal role; and as an associated finding in the biopsy evaluation of other neuromuscular diseases. The question then arises, whether this association is simply coincidental or whether the deficiency occurs secondarily.[12] Furthermore, it stresses the need for a reliable, non-invasive screening test.
2. The question has been put forward whether MAD-deficiency is a disease in itself or not.

I.2 Purine metabolism and MAD in muscle.

The prime function of muscle is to generate force.[22] The performance of muscle was early recognized te be dependent on energy derived from chemical reactions within the tissue.[23] The transducer of chemical energy to mechanical work in muscle is adenosinetriphosphate (ATP). So ATP is the immediate source of energy for muscular contraction.[24,25] If energy is required, ATP is hydrolyzed to adenosinediphosphate (ADP) and adenosinemonophosphate (AMP). This reaction is not dependent on oxygen. The ATP store, however, is in itself very limited and has to be continuously refilled. [26] The sources for ATP-resynthesis are 1. breakdown of creatine phosphate (CP, the Lohmann reaction), 2. anaerobic glycogenolysis, and 3. if oxygen is available, oxidation of pyruvate or fatty acids and oxydative phosphorylation in mitochondria[24] (Table 2). CP acts as a secondary energy store and provides a means for the immediate resynthesis of ATP.[25] CP probably has its greatest importance for contraction during the first few seconds of increased energy demands before other energy-delivering systems (e.g. glycolysis) have had time to adapt. ATP is then very

Table 2 Energy sources for muscular activity (Reaction equations are not complete!)

Short term (anaerobic)sources
creatinephosphate + ADP $\rightleftharpoons$ creatine + ATP
glycogen/glucose + ADP + P_i $\longrightarrow$ lactate + ATP
Long term (aerobic)sources
glycogen/glucose + ADP + P_i + O_2 $\longrightarrow$ H_2O + CO_2 + ATP
fatty acids + ADP + P_i + O_2 $\longrightarrow$ H_2O + CO_2 + ATP

rapidly regenerated through the rephosphorylation of ADP by CP with the aid of creatinekinase.[27]

Interlude 1.

Nucleotides i.e. the constituting elements of nucleic-acids, are composed of a purine- or pyrimidine base, a monosaccharide (nearly always ribose) and phosphoric acid. ATP is a purine nucleotide with the base adenine, the sugar ribose and 3 phosphoric acid groups. ADP and AMP contain two and one phosphoric acid group respectively. Adenine (6-amino-purine) contains a cyclical structure which was called a purine ('purum uricum') because this cyclical structure is the basis skeleton for uric acid.

Nucleosides are called the molecules which consist of a purine or pyrimidine base and a sugar, and carry trivial names corresponding to their base, which in the case of the purine nucleotides will end on -osin, e.g. for adenine the nucleoside's nomenclature will be adenosine.[28]
Figure 2 shows a simplified scheme of purine nucleotide breakdown.

The size of the total adenine nucleotide pool (TAN: ATP + ADP + AMP) can decrease under some physiological conditions, but this can only occur by the breakdown of AMP.[26] Two separate pathways are possible (Fig. 2): deamination to inosinemonophosphate (IMP) or dephosphorylation to adenosine. In skeletal muscle deamination seems to be the main route, whereas in heart muscle both routes are available.[29] Deamination of AMP and thus the liberation of ammonia, is catalyzed by MAD and is essentially an irreversible reaction.

I.3. Purine nucleotide cycle.

In the early twenties ammonia was one of the topics in physiology. Well known investigators as Parnas, Embden, Meyerhof, Lohmann and Meyer, among others, studied the production of ammonia and uric acid. Already two decades before, Richard Burian[30] demonstrated the connection between exercise and an increasing amount of uric acid excreted in the urine although he had no idea about its source. In 1928 Embden[31 33] ascertained the relation between the formation of ammonia and muscular work, and recognized that ammonia production depends on the frequency of muscle stimulation: the higher the frequency the more pronounced the production. The ammonia produced was derived from an adenine nucleotide and Embden et al. succeeded in isolating this compound. Shortly thereafter Parnas[34 36] confirmed these findings and was the first to perceive the possibility of muscle to resynthesize this adenine nucleotide not simply by a reversal of the reaction but via, what he described as, a purine nucleotide cycle ('Kreislauf der Purinnucleotide')! He delineated the chain of reaction as follows:

1. contraction : adenine nucleotide ⟶ IMP + NH_3
2. at rest : IMP + O_2 + amino acid derivate X ⟶ AMP + deaminated product of X.

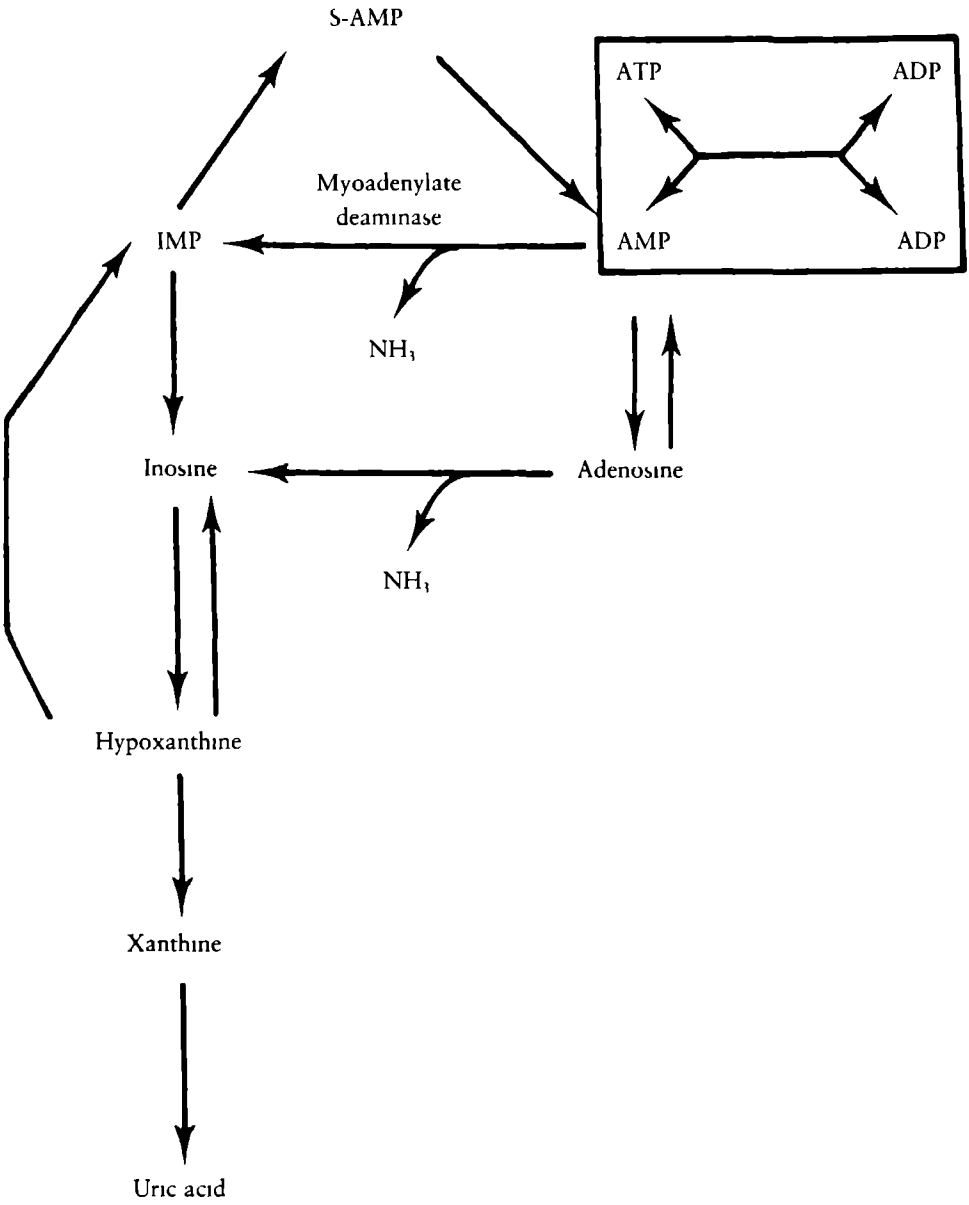

Figure 2. Simplified scheme of purine nucleotide breakdown

The first step in the delineation of the purine nucleotide cycle (PNC, Fig. 3) was made by G. Schmidt,[37] one of Embden's co-workers, in 1928. He succeeded in isolating MAD and drew the conclusion from his experiments that the formation of ammonia from AMP and adenosine (Fig. 2) involves two different deaminases, MAD and adenosinedeaminase (ADA). The other two enzymes which together with MAD constitute the PNC, adenylosuccinate synthetase and adenylosuccinase, were identified in 1955.[38]

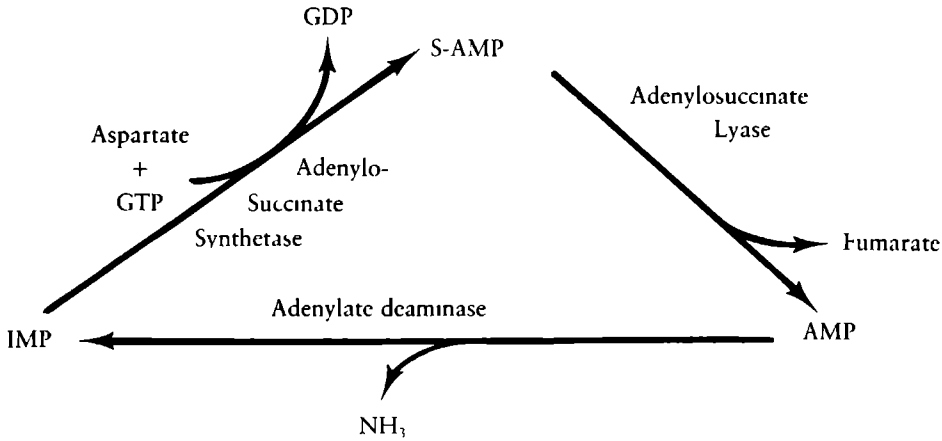

Fig 3 Purine Nucleotide Cycle

The PNC appeared again before the footlights by the elaborate studies of Lowenstein et al.[39] They proposed the following functions for this cycle:

1. regulation of the relative levels of the adenine nucleotides AMP, ADP and ATP. Conversion of AMP to IMP by MAD leads to the formation of ATP from ADP by a readjustment of the adenylate kinase (myokinase) equilibrium: 2 ADP $\leftrightarrows$ 1 ATP + 1 AMP. In this way it serves to maintain a high ATP/ADP ratio.
2. liberation of ammonia from amino acids via aspartate, which thereby makes available the carbon skeletons of amino acids for energy production. A combination of the reactions catalyzed by the enzymes of the PNC and by fumarase, malate dehydrogenase and phosphoenolpyruvate carboxykinase leads to the conversion of aspartate to phosphoenolpyruvate, which can then be oxidized completely.
3. adjustment of the levels of citric acid cycle intermediates.
4. control of phosphofructokinase and hence of glycolysis; this is achieved via effects on the levels of AMP and, possibly, of ammonia, both of which are activators of the enzyme.
5. regulation of phosphorylase b by accumulation of IMP, an activator of the enzyme under conditions when AMP is low and phosphorylase exists largely in the b form.

Ad 1.

The relative levels of ATP, ADP and AMP point to the concept of the 'energy charge' ($EC = \frac{ATP + \frac{1}{2} ADP}{ATP + ADP + AMP}$) as introduced by Atkinson et al.[40] They suggested that the regeneration and expenditure of ATP was controlled by the balance among the concentrations of the adenine nucleotides ATP, ADP and AMP. Atkinson drew a parallel between the adenylate system and an electrochemical storage cell in its ability to accept, store, and supply energy. The adenylate energy storage system may be described as the sum of the reactions:

1. $ATP + AMP \leftrightarrows 2\ ADP$
2. $2\ ADP + 2P_i \leftrightarrows 2\ ATP + 2H_2O$

   $AMP + 2P_i \leftrightarrows ATP + 2H_2O$

The energy charge varies from 0 (only AMP present) to 1 (only ATP present).

Ad 2 and 3.

These possible functions are more complicated because both refer to the oxydative ATP regenerating system, i.e., the PNC is then supposed to work under aerobic circumstances; otherwise the increase in citric acid cycle intermediates would have no effect. Aragon et al.[41] indeed noticed a fourfold increase of fumarate during the first 10 minutes of rat skeletal muscle exercise. Although the replenishment of citric acid cycle intermediates may also occur via other reactions, e.g. catalyzed by alanine-glutamate aminotransferase, glutamate dehydrogenase, pyruvate carboxylase and malic enzyme, with the aid of an adenylosuccinate synthetase inhibitor the authors obtained strong evidence that part, if not all, of the increase in citric acid cycle intermediates observed during exercise was due to the generation of fumarate by means of the purine nucleotide cycle.

In this case, however, the turnover of the PNC will also result in a resynthesis of AMP and thus counteract the initial shift in the adenylate kinase reaction towards ATP production; but because of the low level of adenylosuccinase activity in skeletal muscle as compared to the MAD activity, it seems doubtful that AMP regeneration could keep pace with AMP deamination during intense mucular work.[42] Besides, the key regulatory enzyme controlling the resynthesis of adenylates, adenylosuccinate synthetase, is inhibited by AMP and ADP and, therefore, not likely to be very active during intense muscle stimulation.[43] Some studies[42 69] suggest both limbs of the PNC operate simultaneously during muscle stimulation, while others[44] suggest the two limbs operate in series i.e. AMP ⟶ IMP during muscle contraction and IMP ⟶ S-AMP ⟶ AMP during the relaxation period following muscle contraction. Anyhow, there is no quibbling about the deamination of AMP to IMP during exercise, which is most apparent during anoxic stimulation due to metabolic changes favourable for activation.[45 47]

Ad 4 and 5.

Both proposed functions suggest an involvement of the PNC in the enhancement of glyco(geno)lysis during muscular activity. This would mean that in case of MAD-deficiency, and thus a disrupted PNC, glyco(geno)lysis would take place to a smaller extent than in normals.

I.4. Some characteristics of AMP deaminase

The activity of AMP deaminase, a predominantly extramitochondrial enzyme, is very high in skeletal muscle in comparison with that of all other tissues, including heart and smooth muscles.[19] The highest activities are found in white muscle.[48,49] There are several isoenzymes of AMP deaminase, at least three isoenzymes have been identified on the basis of quite distinct kinetic and immunologic properties.[50-52] Isoenzyme A is found only in skeletal muscle and diaphragm, isoenzyme B is the predominant form in liver, kidney and testes, isoenzyme C is the only form in the heart, and hybrids of isoenzymes B and C are found in brain, lung and spleen.[53]
It is beyond the scope of this study to go into the subject of possible different types of AMP deaminase in red and white muscle, as put forward by Ogasawara et al.[48] and Raggi et al.[49] In case of the deficiency state the enzyme was almost completely absent in all fibre-types.[9,10,21]
The patients lacking the muscle isoenzyme had nevertheless normal levels of AMP deaminase activity in their erythrocytes, suggestive of a hereditary deficiency. Crossmixing studies of normal and MAD-deficient muscle homogenates showed no evidence of an enzyme inhibitor, albeit a serum/plasma inhibitor of MAD does exist and might traverse the muscle membrane.[12,29,54] Rabbit antisera to human MAD failed to react with the AMPD of purified human erythrocytes and other tissues.[55] The deficit is not an artifactual result of freezing damage since fresh and frozen biopsies in the same patient showed equivalent deficits (own experiments and Fishbein's[12]).
All these data indicate a specific deficiency of MAD.

I.5. The consequences of MAD-deficiency.

The preceding statement find a logical continuation in the question: is there a relation between the complaints of exertional myalgia and a disturbance of the purine metabolism as a consequence of deficient MAD-activity?
In attempts to answer this question two approaches may be useful: 1. the analysis of changes of purine nucleosides and bases in the venous effluent of the working muscle and 2. the analysis of changes of purine nucleotides, nucleosides and bases in the exercised muscle itself.
Burian[30] recognized already in 1905 an increase in the 'nonuric acid purine' excretion in urine during muscular exercise and in the period immediately following. Although the origin of these purines remained obscure to him, he attributed the endogenous production of uric acid and other purines in man to muscular activity. The magnitude of the increase in the purine excretion depended on the performance delivered. Cathcart et al.[76] in 1907 and Saiki et al.[77] in 1932 confirmed these findings.[78]
Because of the relatively recent recognition of MAD-deficiency as the cause of a metabolic myopathy, literature with regard to the efflux of purine nucleosides and bases under this condition is limited. In 1983 Patterson et al.,[79] and in 1984 Joosten et al.[15] reported on subnormal amounts of hypoxanthine produced during forearm exercise in MAD-deficiency. The measurement of plasma purines as a marker for ATP

degradation in human muscle diseases appeared to be a promising method. Soon after these first reports others recognized its value to gain insight in diseases as myophosphorylase- and carnitinepalmitoyltransferase deficiency.[57,80]
The second approach, i.e. the assay of the metabolites in muscle itself, found its principal investigators in Sabina et al.[4,44] They reported that the skeletal muscle of MAD-deficient patients has a lower capacity for energy production than does the muscle from non-MAD-deficient patients and controls. From their findings they suggested that the PNC has a previously unrecognized function in providing a mechanism for preserving the purine nucleotide content of exercising muscle through accumulation of IMP, a non-diffusible nucleotide, which can be used to rapidly restore the ATP pool during periods of rest.[4,44]

I.6. Screening in MAD-deficiency.

The major source of ammonia in working muscle is the ammonia liberated during the conversion of adenosine monophosphate to inosine monophosphate by the enzyme MAD (Fig. 2).[39] Based on this, Fishbein et al.[1] used the lactate/ammonia ratio in a forearm test to screen for MAD-deficiency. In their first study on this disease, they inflated the sphygmomanometer cuff around the upper arm halfway diastolic- and systolic blood pressure as to reach ischaemia and gave their patients a sponge to squeeze. This procedure impairs interindividual comparisons concerning the amount of work delivered. Others[4] instructed the patient to rapidly open and close his fist until muscle fatigue set in or asked the subject to contract maximally for 1 minute.[57] So with these divergences in screening methods it seems appropriate to ask: which type of exercise should be imposed in order to maximally stimulate MAD-activity and to adapt this test to a sensitive and specific tool in screening for MAD-deficiency. A prerequisite to answer this question is standardization. Munsat[56] developed a standardized ischaemic test for screening defects in the glyco(geno)lytic pathway using a handgrip dynamometer. However, no attempt was made to find out which force level and frequency of contractions resulted in blood concentrations of lactate that optimally discriminated between patients and controls. Mutatis mutandis the same holds true for the ammonia produced. As mentioned before, there seems to be a relation between work rate and ammonia production: the higher the frequency of muscle stimulation, or the higher the muscle force exerted during contraction, the more pronounced the formation of ammonia.[31,33,66,68] Besides, fast twitch white muscle produces more ammonia than red muscle.[34,36,64,65]

Interlude 2.

Skeletal muscles are composed of different types of fibres, which can be conveniently classified into three categories on the basis of three criteria:[58,59]

1. *the contraction time of the fibre: slow twitch or fast twitch,*
2. *the glycolytic capacity,*
3. *the oxidative capacity.*

Using a histochemical approach to the classification of fibre types and in accordance with the findings of Peter,[58] Dubowitz and Brooke[60] the three fibre types are:

Type I : slow-twitch oxidative, formerly designated as slow-twitch red. These fibres rely predominantly on the aerobic metabolism for the production of ATP.

Type II A : fast-twitch oxidative-glycolytic, formerly designated as fast-twitch red.

Type II B : fast-twitch glycolytic, formerly designated as fast-twitch white. These fibres rely mainly on the anaerobic metabolism for the production of ATP.

The nerves innervating muscle fibres have their origin in the cell bodies lying in the anterior horn of the spinal cord. The neuron which originates in a single anterior horn cell will branch to supply many muscle fibres. Functionally, the anterior horn cell, its axon and the muscle fibres supplied by it, behave as one unit, the motor unit.[60] Burke et al.[61] have shown in cats by direct stimulation of the anterior horn cells the presence of three kinds of motor units, corresponding with the type I, type II A and type II B muscle fibres and designated as slow-twitch, fast-twitch fatigue resistant, and fast-twitch easily fatigued respectively.[60] Resistance to fatigue was closely related to the activities of the oxidative enzymes.[62] A similar classification of human skeletal muscle motor units has been made.[63]

A requisite for MAD-activity is, of course, a certain amount of substrate, i.e. AMP. The formation of AMP implies an imbalance between ATP utilization and ATP resynthesis, as is the case in ischaemic exercise. When oxygen and fuel supply can keep pace with the demand as in the steady state situation of aerobic metabolism, oxidative rephosphorylation of ADP will occur, thereby preventing a dismutation to AMP and ATP.

When one takes into account that the highest activities of MAD are found in type II fibres, it is not amazing to find in exercising humans an exponential rise in blood ammonia with increasing work loads.[69-71] This is in accordance with the assumption of an ordered recruitment pattern of muscle fibre types: at low work loads predominantly slow-twitch fibres will be used, and ammonia production will become more evident at higher loads when more fast-twitch fibres are recruited.[31,59,72,73] Despite these plausible considerations, which suggest a consistent relation between work intensity and ammonia production, the increase in ammonia is known to vary widely among individuals, even at the same relative workload.[66,68]

This variability in ammonia production may be related, among others, to the wide variability in fibre type composition among individuals, and therefore made it worthwhile, in connection with the refinement of the screening method, to perform experiments with different work intensities in order to find the highest ammonia values.

An important component of the energy at high work loads is derived from anaerobic glycolysis, which implies, among others, an accumulation of lactate. Although Fishbein[12] found a linear relationship between the ammonia and lactate produced in a more or less ischaemic exercise test, this linearity in itself is not important for

screening purposes. Measuring the lactate concentration offers the possibility to estimate the amount of work performed under anaerobic conditions.

Improvement of screening in exertional myalgia implies muscle contractions in such a manner as to produce high levels of lactate and ammonia. In order to achieve these requirements it seems logical to impose ischaemia and a high work load. Standardization of the contraction force and frequency is obligatory.

I.7 Genetics

The mode of inheritance of MAD-deficiency has not been definitely established. X-linked recessive[81] as well as autosomal dominant inheritance[19] have been suggested, but most authors, including Fishbein[12] and Mc Kusick,[82] consider it to be autosomal recessive. As mentioned before in the review of the study of Hayes et al.[11] the possibility of heterozygotes for MAD-deficiency also being symptomatic has not been carefully evaluated and is mostly founded on circumstantial evidence. Only in one study we found a carrier evaluation.[21] It is quite difficult to identify heterozygotes as outlined by Fishbein et al.[21] because of:

1. the variation of muscle enzymes with fibre type (e.g., the highest MAD-activity is found in type II B fibres);
2. the non-specific decrease of enzyme activity with disuse, disease or specimen mishandling;
3. the inadvertent inclusion of carriers in the control population.

Fishbein et al.[21] measured MAD levels in six putative heterozygotes, both male and female, four of whom complained of exertional myalgia, and found in all six muscle specimens MAD-activity of less than 40% of the normal mean value after correction for their fibre-type distribution. Hence they concluded that the heterozygous state does exist and that the inheritance pattern is autosomal recessive.

I.8 The intents.

In the preceding paragraphs of this chapter the questions and controversies which exist with regard to myoadenylate deaminase deficiency have been outlined. As such these issues are the subject of this study.

In each of the following 6 chapters an answer is formulated to a specific question that has arisen. Previous to the enumeration of the questions it seems wise to give a synopsis of the problems encountered. A prerequisite to study a newly described disorder is the delineation of this disturbance in order to distinguish it from other pathologic entities. Only then is it possible to perceive a specific underlying pathophysiological mechanism, if any.

So the first implication is the necessity of developing a reliable screening method, certainly when a discriminative set of symptoms and/or signs is not on hand. Once the deficiency is easily diagnosed by means of a screening test, the next step is to look for a relationship between the complaints, i.e. exertional myalgia, and a disturbance in

purine metabolism as a consequence of the deficiency and as the biochemical base of the pathophysiology. At last, if a disease is supposed to be inherited, one wonders about its mode of inheritance and frequency of occurrence.
So the aims of this thesis concerning the deficiency of myoadenylate deaminase were to find answers to the following questions:

1. The development of a non-invasive screening method in order to minimize the number of muscle biopsies needed.
2. Does standardization improve the specificity and sensitivity of the screening test?
3. What are the biochemical consequences of MAD-deficiency concerning purine metabolism as measured in the venous effluent of exercising muscle?
4. What are the biochemical consequences of MAD-deficiency concerning purine metabolism as measured in muscle itself?
5. What are the genetics of the deficiency on base of the screening of families of probands? Do eventually newly diagnosed patients in these families have complaints?
6. What is the frequency of occurrence of MAD-deficiency in muscle biopsies of patients without exertional myalgia (retrospective) and in patients with exertional myalgia which are screened with the standardized ischaemic exercise test (prospective)? These frequencies are important for the clinical relevance of the deficiency.

All the patients in this study are listed in the Appendix.

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Chapter II

THE RELATION BETWEEN BLOOD LACTATE AND AMMONIA IN ISCHEMIC HANDGRIP EXERCISE

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SUMMARY

Subjects with myopathies associated with certain enzyme defects often show abnormal concentrations of lactate (LA) and ammonia (NH_3) in blood after ischemic exercise. Myoadenylate deaminase (MAD) deficient patients produce only small amounts of NH_3, whereas LA rises to normal levels. On the other hand, patients with certain enzyme deficiencies in the glyco(geno)lytic pathway show the opposite. However, the concentrations in blood are dependent on the exercise performed. Standardization of tests for screening purposes, therefore, is necessary. For ischemic contractions, experiments were performed to find the optimal combination for force and frequency, using the highest LA and NH_3 concentrations in blood as criteria. Eleven healthy subjects performed ischemic isometric contractions with a handgrip dynamometer at frequencies of 30 and 50/min^{-1} and force levels of 50%, 65%, and 80% of maximal voluntary contraction force (MVC). A combination of 30/min^{-1} and 80% MVC was found to give the best results.

INTRODUCTION

In 1978, Fishbein et al.[4] discovered a new muscle disease associated with weakness and cramping after exercise myoadenylate deaminase (MAD)deficiency. MAD catalyzes the irreversible deamination of adenosine monophosphate (AMP) to inosine monophosphate (IMP). Resynthesis of AMP occurs by the sequential action of adenylosuccinate synthetase and adenylosuccinase. The reaction chain thus constituted is called the purine nucleotide cycle (Fig 1). Necessary for the purine nu-

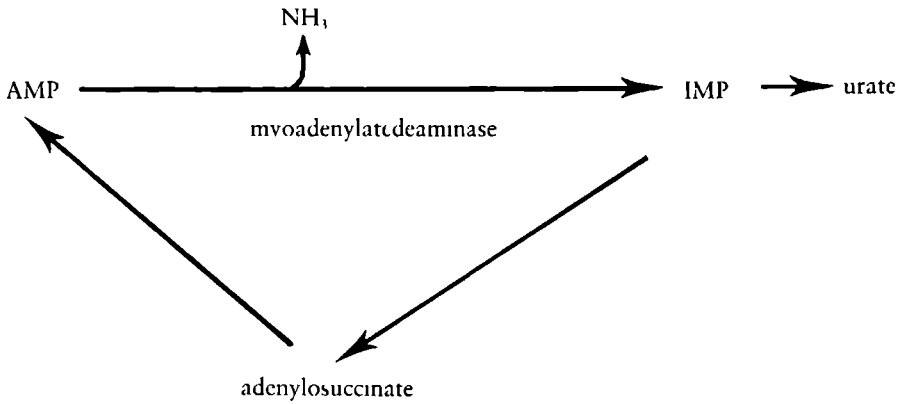

Figure 1 Main characteristics of the purine nucleotide cycle Modified after Sahlin et al [13] See text for explanation

cleotide cycle are aspartate and guanosine triphosphate (GTP), while fumarate, guanosine diphosphate (GDP), organic phosphate, and ammonia (NH_3) are formed. The purine nucleotide cycle is known to be active during exercise and especially during ischemic exercise [8 11 15 16] Based on this fact, Fishbein et al.[4] devised a simple screening test for subjects suspected of MAD-deficiency. The subjects were asked to squeeze a sponge for 1 or 2 minutes. Lactate (LA), as an indicator for glycolytic activity, and NH_3 as an indicator for MAD-activity, were measured in blood, which was sampled after the exercise period. In 'normal' subjects, a positive correlation was found between the LA and NH_3 produced. Subjects with MAD-deficiency showed very little NH_3 production relative to the production of lactate.

The relationship between NH_3 and LA produced during exercise not only reveals information about a defect in the purine nucleotide cycle, but several enzyme deficiencies in glyco(geno)lysis can also be detected. Among these are the phosphorylase, phosphofructokinase, phosphoglycerate mutase, phosphoglycerate kinase, and lactate dehydrogenase deficiencies (see DiMauro et al.[3] for references). Almost no LA can be formed, whereas NH_3 formation is not inhibited because the purine nucleotide cycle is not affected

It can be deduced that the defects described will appear clearest during heavy anaerobic muscle contractions. These exercise tests for screening patients vary from

one clinic to another and are not strictly standardized. Fishbein et al.,[4] in their first paper on this disease, gave their patients a sponge to squeeze. Sabina et al.[12] instructed the patient to rapidly open and close the fist until muscle fatigue sets in, whereas Hayes et al.[5] asked the subjects to squeeze an inflated sphygmomanometer cuff for 2 minutes. Brooke et al.[1] asked their subjects to contract maximally during 1 minute. Munsat[10] developed a standardized ischemic test for screening defects in the glyco(geno)lytic pathway using a handgrip dynamometer. A fixed frequency of 60 contractions per minute was imposed, and the workload was varied by attaching different springloads. However, no attempt was made to find out which force level and frequency of contractions resulted in blood concentrations of LA that optimally discriminated between patients and controls.

The purpose of this study is to develop a standardized handgrip test with an optimal combination of force and frequency to screen for MAD-deficiency as well as for several glyco(geno)lytic enzyme deficiencies. Criterion for the optimal combination are the highest concentrations of LA and NH_3 after exercise. Ischemic exercise was chosen because the LA[10] and NH_3 concentrations reach higher levels than they do in nonischemic exercise.

MATERIALS AND METHODS

Subjects. Eleven healthy subjects without neuromuscular symptoms participated in the study. There were 4 females, with a mean age of 26(22-31) years, a mean weight of 60(57-65)kg, and mean height 167(159-172)cm, and 7 males, mean age 38(24-54)years, mean weight 78(69-93)kg, and mean height 184(177-198)cm. The subjects performed isometric handgrip tests with the dominant hand at contraction frequencies of 30 and 50/min^{-1} and force levels of 50%, 65%, and 80% of the maximal voluntary contraction force (MVC). The tests were interspaced by at least 1 week. The force and frequency levels were obtained from a preliminary study in which it was shown that a test duration of 1-3 minutes was a prerequisite to be sure that the anaerobic pathway is heavily taxed, and frequencies over 50/min^{-1} were too high to execute the test correctly.

Procedure. The subject was seated on a chair, with the forearm horizontally on the level of the handgrip dynamometer (Fig. 2). The handgrip width was adjusted to the size of the hand: the medial phalanx of the middle finger had to be perpendicular to the proximal phalanx. Next, the MVC was determined. The highest value of three maximal contractions, interspaced by 1 minute, which could be maintained for at least 3 seconds, was taken as the MVC. After a rest of 10 minutes, a Teflon catheter (Abbocath 18G. ø 0.9 mm, Abboth Ireland Ltd, Sligo, Republic of Ireland) was inserted in an antecubital vein, and 3 minutes later, 10 ml of blood was sampled to obtain the resting values of LA and NH_3. Two minutes later, a cuff around the upper arm was inflated to 200 mm Hg, and the subject was asked to start exercising at a predetermined frequency and force level. Each subject performed seven tests, including a preliminary test. The following order of the different combinations of force and

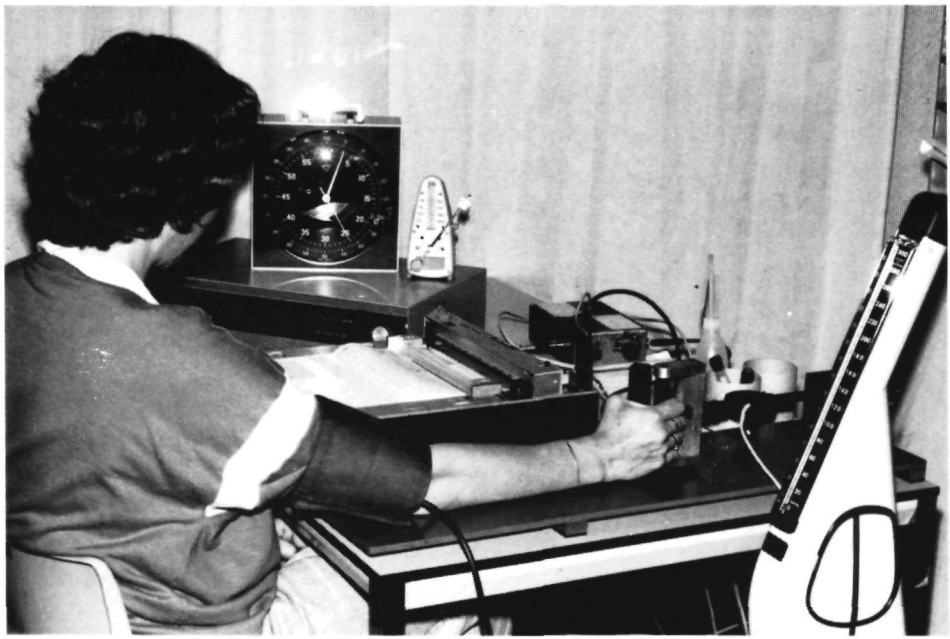

Figure 2. The experimental setup: handgrip dynamometer, recorder, metronome.

frequency levels was randomly assessed per subject. At the moment the subject could no longer deliver any force, despite encouragements from the leader of the experiment, the test was stopped. Immediately after deflation of the cuff, a blood sample was taken, and this was repeated after 3, 5, and 7 minutes. The highest levels for LA and NH_3 after exercise in all 77 experiments were reached for LA 29 times immediately after exercise, 45 times at 3 minutes, and 3 times at 5 minutes. For NH_3, the data are 19 times immediately after, 32 times at 3 minutes, 23 times at 5 minutes, and 3 times at 7 minutes. LA and NH_3 peaked 47 times at the same moment. In the other cases, the time difference in the appearance of the peak was generally not more than 3 minutes. In general, the subjects who showed the highest value earlier or later did this after all experiments, but not exactly at the same time.

Physiologic Variables and Apparatus. The force was measured with a strain-gauge dynamometer. The amplified signal was continuously recorded on a strip chart recorder and was presented to the subject. The required force level was indicated on the recorder with a solid line. Frequency was indicated by a metronome at frequencies of 30 or 50 contractions per minute. To obtain an indication for the amount of muscle work delivered, the total impulse (TI), that is, the sum of the integral of force and time (in kgf x sec) of all contractions, was measured with a Kipp BC-1 integrator (Kipp, Delft, The Netherlands). The number of contractions and the time until exhaustion were derived from the registration of the stripchart. Blood was analyzed for NH_3 on a Multistatt III centrifugal analyzer (Instrumentation Laboratory, Harrow, Middlesex, UK), with an adaptation on the enzymatic UV-test, as described by Da Fonseca-

Wollheim and Maigatter.[2] LA was determined enzymatically, with minor modifications, after Henry et al.[6]

When appropriate, statistical comparisons between the blood concentrations after exercise were made by three-way analysis of variance (ANOVA-force level effects, frequency effects, and interindividual differences). A $P < 0.05$ level of significance was used for these comparisons.

RESULTS

MVC in the first experiment was significantly lower than the MVC in repetitions. The mean MVC for all subjects during the six successive experimental days were 100%, 110%, 114%, 112%, 112%, and 112%, respectively.

The individual LA and NH_3 concentrations measured at rest in all six test situations ranged from 808 ± 49 to 1272 ± 102 μmole.liter^{-1} (mean ± SE) and 22.5 ± 2.8 to 47.2 ± 2.2 μmole.liter^{-1},respectively. The highest concentrations measured after the exercise period are presented in Table 1 for each combination of frequency and force level.

Analysis of variance revealed that no significant differences existed between the frequencies for maximum lactate and ammonia. However, a significant difference was found between the force levels for LA ($P < 0.05$). The highest force level showed the highest lactate concentration. For NH_3, no such difference could be found. The same applied when the delta value (maximal concentration – concentration at rest) for LA (Δ LA) and NH_3 (Δ NH_3) were taken into account.

In Table 1 is also presented the time to exhaustion for each combination of force and frequency. Frequency and force level were the main determinants of the number of contractions and the time to exhaustion. ANOVA indicated that TI was dependent on the frequency ($P < 0.001$), TI being higher at 30.min^{-1} than at 50.min^{-1} (Table 1). No differences for TI between the force levels could be found ($P > 0.05$).

Table 1 Maximum ammonia (NH_3) and lactate (LA) concentrations in blood (μmol l^{-1}), total impulse (TI, kgf × sec), and time to exhaustion (ET, seconds) for each combination of force (% MVC) and frequency (per minute) during ischemic isometric handgrip contractions in normal subjects (n = 11, mean ± SEM)

	Percent MVC		
	50	65	80
Frequency			
30			
NH_3	169 ± 15	169 ± 19	174 ± 17
LA	5365 ± 491	5640 ± 491	6031 ± 594
TI	1663 ± 155	1696 ± 104	1652 ± 135
ET	159 ± 6	112 ± 5	89 ± 4
50			
NH_3	159 ± 17	162 ± 18	166 ± 17
LA	5502 ± 401	5395 ± 545	6605 ± 449
TI	1320 ± 89	1303 ± 85	1309 ± 108
ET	116 ± 4	86 ± 3	68 ± 3

DISCUSSION

In the first testing of the subject, the MVC was significantly lower than in the remaining tests. Kroll[7] described the same phenomenon: 3 weeks after the first determination of the MVC, the MVC appeared to be 8-15% higher. The most plausible explanation is a motor learning effect, as it is very unlikely that any physiologic variable is altered. The lower MVC in the first visit has not influenced the results, because the following order of the experiments per individual was randomized prior to the experiment. In practice, however, it may be worthwhile to perform the MVC test twice, interspaced by a resting pause of several minutes at the first session, to avoid underestimation of the MVC. Time to exhaustion appears to be almost completely determined by the force level and frequency of repetition. Interindividual differences, however, still accounted for a significant part of the variance. The MVC was not related to the time to exhaustion, which confirms the findings in continuous isometric exercise.[14]

As patients with an enzyme deficiency in the glyco(geno)lytic pathway or in the purine nucleotide cycle are known to make lesser amounts of LA and NH_3 during muscle work, it is essential for screening purposes that the control subjects investigated do have the highest possible concentrations of LA and NH_3. The highest LA concentrations in blood are found when a force level of 80% MVC was imposed (Table 1). Furthermore, there is a tendency for the NH_3 levels to be highest also at 80% MVC (Table 1). The findings for the LA levels are consistent with data from Wahren,[17] who used intermittent isometric exercise with a handgrip dynamometer. The highest concentration of lactate was found with the highest force imposed. For the purpose of a screening test, these data indicate that the force level to be chosen should be 80% MVC.

It is not easy to determine the optimal frequency at which to perform the test. There was a tendency for the maximal NH_3 concentrations to be higher at the frequency of 30 contractions per minute as compared with the frequency of 50 contractions per minute (Table 1). Moreover, some subjects had difficulties in maintaining the frequency of 50 contractions per minute, whereas the frequency of 30 contractions per minute could be fulfilled without difficulties. Therefore, the frequency of 30 contractions per minute seems to be most appropriate for a standardized procedure.

It is difficult to explain why NH_3 was not significantly higher when the muscle 'work' was at its hardest. As comparative literature data for these sorts of tests are not available, it can only be speculated that the production of NH_3 is already maximal at the lowest exercise level used here. Another explanation might be that NH_3 is slowly released from the muscular tissue (for references see Mutch and Bannister[11]), showing a peak level after exercise that is not representative for the production in the muscle.

The data for TI did not add any extra information to that obtained from the relation between performance and LA or NH_3 (see Table 1). This was probably due to the relatively small range of force levels used and the large inter- and intraindividual differences. Measurement of TI in a screening test using the force levels and frequencies used here does not seem to be necessary, at least in a group of healthy subjects,

such as those used here. The future might show that a small TI is an indication that the subject probably suffers from a defect in the glyco(geno)lytic pathway.

For the chosen combination of force level and frequency (respectively, 80% MVC and 30 contractions per minute), the relationship between the highest Δ NH_3 and Δ LA is given in Fig. 3. In this figure, data of LA and NH_3 are included from six McArdle patients[9] and eight MAD-deficient patients[4] observed in the Department of Neurology. The patients performed the exercise test with the same combination of force (80% MVC) and frequency ($30/min^{-1}$) as the controls. It should be noted that their resting values of LA and NH_3 are not essentially different from those of the control subjects. These data suggest that this standardized test might be valuable in screening subjects with enzyme deficiencies in the glyco(geno)lytic pathway.

In summary, for screening subjects suspected of either an enzyme deficiency in the glyco(geno)lytic pathway or a MAD-deficiency, an ischemic isometric handgrip test is proposed with a contraction frequency of $30.min^{-1}$ and a force level of 80% MVC. A preliminary reference figure is given for the relationship between LA and NH_3.

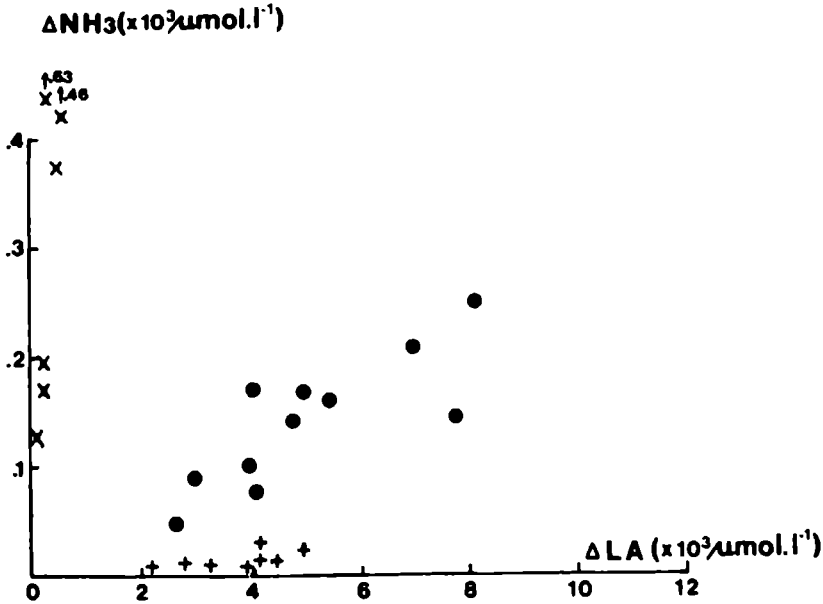

Figure 3. The highest increase (Δ), compared with rest, of LA and NH_3 after ischemic isometric handgrip exercise with 80% MVC and 30 contractions per minute (11 control subjects, •; MAD-deficient patients, +; McArdle patients, x). Regression lines for controls: $\Delta NH_3 = 0.027 \Delta LA + 1$; $\Delta LA = 26.64 \Delta NH_3 + 1525$; $r = 0.83$.

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Chapter III

IMPROVEMENT OF SCREENING IN EXERTIONAL MYALGIA WITH A STANDARDIZED ISCHEMIC FOREARM TEST

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SUMMARY

An ischemic forearm test with simultaneous measurement of both lactate and ammonia can be used as a screening method for myoadenylate deaminase (MAD) deficiency and for various glyco(geno)lytic defects. A standardized and a nonstandardized test have been compared in a group of 186 patients with exertional myalgia. Standardization of the ischemic forearm test has led to greater yields of both lactate and ammonia in venous return blood of patients and controls. The sensitivity of the proposed test procedure in detecting MAD-deficient patients was 100%, whereas the specificity amounted to 98.8% among exertional myalgia patients.

INTRODUCTION

With the development of a staining procedure for myoadenylate deaminase (MAD) in muscle biopsies, it became clear that the deficiency state for this enzyme was not rare at all.[5] Estimations of its occurrence ranged from 1% –2% in leftover biopsy material[5,7,16] to 8.3% in exertional myalgia patients,[8] making it the most common of all known enzyme deficiencies of skeletal muscle.[4]

The clinical symptoms of this metabolic myopathy are ill-defined and encompass exercise-induced muscle pain and soreness, fatigue, and cramps. This set of complaints, however, can have a variety of causes, including several metabolic disorders. The high prevalence, in combination with the nonspecific clinical symptoms of MAD-deficiency, illustrate the need for a reliable screening test. However, several reports in the literature have described pitfalls in the ischemic forearm test.[1,8]

Ammonia is liberated during the conversion of adenosine monophosphate (AMP) to inosine monophosphate (IMP) by the enzyme MAD. This reaction is the major source of ammonia in working muscle.[9] Based on this, Fishbein et al.[3] used the lactate/ammonia ratio in a forearm test to screen for myoadenylate deaminase deficiency. Patients with MAD-deficiency have a high lactate/ammonia ratio in this test, whereas patients with glyco(geno)lytic defects (e.g., McArdle's disease) invariably have a low ratio. A major problem in the interpretation of this screening test often lies in the low amount of lactate and ammonia produced by the patient during exercise. Standardization of the contraction force and frequency[17] could be an answer to this problem.

This article compares the nonstandardized ischemic forearm test with a standardized version of the test in screening patients with exertional myalgia. Biochemical data are presented on several neurologic patient groups. Various ways of expressing the biochemical data have been studied in order to find the best method to discriminate between MAD-deficient patients and others.

MATERIALS AND METHODS

Subjects. Four groups of patients were subjected to an ischemic forearm test (Table 1). The patient groups 1a and 4a did the nonstandardized exercise test, and the patients in the groups 1b, 2, 3, and 4b did the standardized exercise test. The patients in group 1 had visited the outpatient ward of the hospital during 2 years of study with exercise-related muscular complaints or with muscle pain, soreness, fatigue, or cramps. Neither MAD-deficient nor McArdle patients have been included in group 1. The MAD-deficient group contained six patients in whom the defect was first discovered in a muscle biopsy. Three patients resulted from a systematic search with the exercise test among exertional myalgia patients. The diagnosis in all cases in this latter group was confirmed enzymatically, as well as histochemically, in a muscle biopsy. McArdle's disease was confirmed by the absence of both phosphorylase protein and phosphorylase enzymatic activity in all patients. The control group consisted of healthy volunteers (students and hospital personnel). All patiens and

Table 1 Description of patient groups

Group 1	Exertional myalgia group	
	(1a) Nonstandardized test	n = 116, 71 men, 45 women, mean age 28 years, range 4-71 years
	(1b) Standardized test	n = 70, 33 men, 37 women, mean age 34 years, range 12-58 years
Group 2	MAD-deficient group	n = 9, 9 men, mean age 40 years, range 14-66 years
Group 3	McArdle group	n = 5, 1 men, 4 women, mean age 20 years, range 8-27 years
Group 4	Control group	
	(4a) Nonstandardized test	n = 32, 15 men, 17 women, mean age 28 years, range 21-48 years
	(4b) Standardized test	n = 22, 13 men, 9 women, mean age 32 years, range 21-55 years

controls gave their informed consent.

Groups 1b an 2 may be considered to be a representative sample of patients for whom the standardized ischemic forearm test is clinically indicated.

Ischemic Forearm Test. Preliminary experiments indicated higher lactate and ammonia production in cases of ischemic exercise as compared to nonischemic exercise. Ischemic exercise has been used in this investigation.

The nonstandardized test. Blood samples for the determination of ammonia and lactate were drawn from the antecubital vein before exercise and at 0, 2, and 4 minutes after exercise (Fig. 1). During exercise, a sphygmomanometer cuff, inflated well above the systolic blood pressure, was around the dominant upper arm of the subject. The cuff was deflated immediately after the exercise period. All subjects performing the test were asked to open and close the fist of their dominant arm vigorously for 2 minutes.

The standardized test. Isometric handgrip contractions till exhaustion at 80% maximal voluntary contraction force and a rate of 30/min were used, as described previously.[17] Blood samples were drawn from the antecubital vein before the exercise and at 0, 3, 5, and 7 minutes thereafter (Fig. 1). Analytical techniques for the measurement of lactate and ammonia have been described elsewhere.[17]

Classification as MAD-deficient or Non-MAD-deficient. Using discriminant analysis, a criterion has been developed for classifying exertional myalgia patients as MAD-deficient or non-MAD-deficient. Three discrimination lines, calculated according to Rao,[13] are presented in Fig. 2. The distribution of the ammonia production in both groups is the first of three factors that are important for the calculation of the discrimination lines. The second factor that should be considered is the prevalence or prior probability of belonging to either one of the groups. In our patient group, the prevalence was 4.1% (3 MAD-deficient patients among 73 patients with exertional myalgia). For this reason, all calculations were carried out using prior probabilities around this value (i.e., 1%, 4.1%, and 8.2%). As this study may not be regarded as an accurate prevalence study, a wide prevalency range has been taken into account. The third factor is the so-called 'loss,' in case a patient is misclassified. If a MAD-deficient patient is classified as non-MAD-deficient, the loss exists as the time wasted to find another diagnosis. If a control is classified as a MAD-deficient patient, the loss exists as an unnecessary biopsy. The loss in case of a misclassified control is arbitrarily set at 1. In case we assume the misclassification of a non-MAD-deficient patient equally

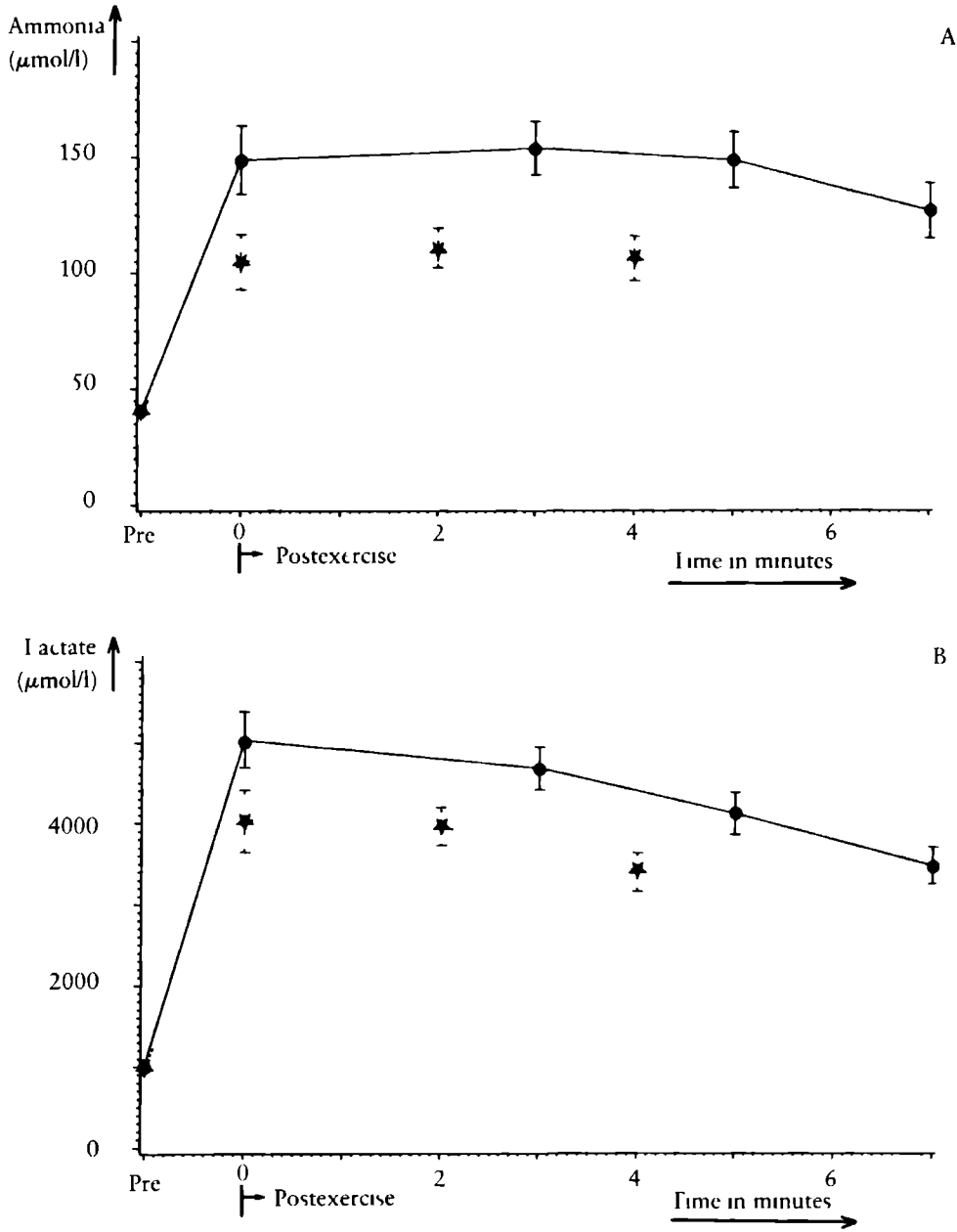

Figure 1 Mean lactate and ammonia production (± 2 SEM) in patients (controls plus exertional myalgia group) for the standardized (●) and the non standardized (★) forearm test
(A) Ammonia, (B) Lactate

serious as the misclassification of a MAD-deficient patient, the loss, L = 1, applies. The loss in case of missing a MAD-deficiency, however, is more serious than the loss in case of a false-positive result. For this reason, loss values of I = 10 and L = 100 have been used Still higher values for L are not considered, because the number of

false-positive controls has to remain acceptable. The choice of L will be discussed below. Unfortunately, there is no simple alternative method for discriminating between MAD-deficient and non-MAD-deficient patients. The only possibility, and at the same time the gold standard, is the muscle biopsy. As indicated in Fig. 2, we did a muscle biopsy on 46 of the 106 patients and controls included in our study (43%). Data on the sensitivity and the specificity of the test will be presented.

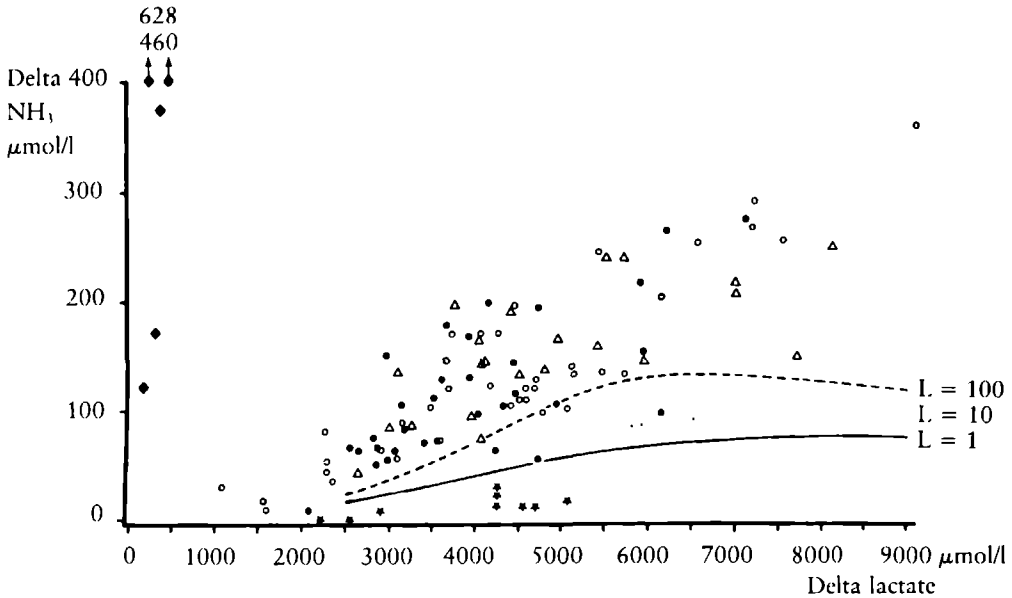

Figure 2. Plot obtained after retransformation of log (delta) values into delta values, giving the decision lines for L = 1, 10 and 100. (★) MAD-deficient patients, (0) exertional myalgia patients, (◆) McArdle patients, (∧) controls. Closed symbols indicate the patients that were biopsied.

RESULTS

Standardization of the Screening Test. Figure 1 shows the results of the ischemic forearm test carried out by patients with exertional myalgia and by controls (groups 1 and 4). The standardization procedure gave considerably higher blood levels for lactate and ammonia ($P < 0.05$). For this reason, only the results obtained with this standardization procedure are presented below.

Physiologic Data. The total impulse (in kg.min) expended by controls, exertional myalgia patients, and MAD-deficient patients without an associated neuromuscular disorder was, respectively, 31.5 (± 4.8), 30.1 (± 8.8), and 30.3 (± 7.6), whereas their endurance time in seconds was, respectively, 87.5 (± 8.4), 101.8 (± 24.4), and 91.3 (± 14.3). These figures are related to men older than 15 years. A comparison between women was not possible due to the lack of female MAD-deficient patients. Two-way analysis of variance revealed no statistically significant differences between the dif-

ferent groups, although the endurance time tended to be longer in the exertional myalgia group in comparison to the control group (P = 0.09).

Blood Levels for Lactate and Ammonia. Mean pre- and postexercise levels of lactate and ammonia are shown in Table 2. Within one group, interindividual differences exist in the lactate and ammonia concentrations after exercise. Some patients had maximal blood levels at t = 0, immediately after exercise, whereas others reached maximal levels later on (Table 3).

Table 2. Mean lactate and ammonia levels (μmol/liter) for the various diagnostic groups performing the standardized test.

	Lactate at time (min)				
	Pre	0	3	5	7
Exertional myalgia	1021	4845	4548	3963	3339
(n = 70)	(297)*	(1605)	(1233)	(1214)	(991)
MAD-deficient	1001	4865	3910	3195	2849
(n = 9)	(326)	(1082)	(892)	(1081)	(1039)
McArdle	906	1100	890	1052	1094
(n = 5)	(203)	(228)	(115)	(103)	(87)
Control	1025	5672	5177	4712	4168
(n = 22)	(260)	(1814)	(1313)	(1233)	(1084)
	Ammonia at time (min)				
	Pre	0	3	5	7
Exertional myalgia	42	143	150	142	120
(n = 70)	(11)	(74)	(56)	(59)	(53)
MAD-deficient	46	54	56	53	51
(n = 9)	(8)	(8)	(8)	(6)	(7)
McArdle	47	399	200	142	107
(n = 5)	(13)	(204)	(37)	(35)	(37)
Control	38	167	170	175	162
(n = 22)	(11)	(58)	(47)	(47)	(45)

* Standard deviation in parentheses

Table 3. The number of exertional myalgia patients at the time of their respective maximal lactate and ammonia levels.

Time of maximal lactate level	Maximal NH_3 level at				
	t = 0	t = 3	t = 5	t = 7	
t = 0	24	11	9	0	44
t = 3	6	13	4	1	24
t = 5	0	0	2	0	2
t = 7	0	0	0	0	0
	30	24	15	1	70

Results of Discriminant Analysis. Three ways of analyzing the ammonia and lactate data have been used.(1) Delta value maximal postexercise value minus the preexercise value.(2) Relative values: maximal postexercise value/preexercise value.(3) Integrated values: integrated value of postexercise minus preexercise concentrations. For statistical reasons, the data were also analyzed after a logarithmic transformation. There are two criteria to decide which way of plotting gives the best results first, the percentage of misclassifications in both the MAD-deficient and the non-MAD-deficient groups (i.e., the sensitivity and the specificity of the test), and second, the posterior probabilities. This is the probability of belonging to one of the two groups given the outcome of the measurements. The posterior probability with respect to correct classification has been calculated for each patient (Table 4). The value for the posterior probability should ideally be close to one. It is not influenced by the value of the loss factor L

Table 4 shows that log (delta) values and log (integrated) values both give good results for the posterior probabilities with all prevalency values considered. The percentage of misclassifications in the MAD-deficient and the non-MAD-deficient groups (i.e., sensitivity and specificity of the test) appear to be similar for both ways of calculating. As will be discussed, it was decided to use the log(delta) values in practice. Using log(delta) values and the L = 10 line, the specificity of the test is 98 8% and the sensitivity amounts to 100%, as can be deduced from Table 4. Figure 2 shows the discrimination lines thus obtained at three L values. In the figure, the log(delta) values have been retransformed to delta values.

Table 4 Mean posterior probabilities and percentage misclassifications

Prevalence for MADD	Mean posterior probability					
	0 01		0 041		0 082	
	Non MADD	MADD	Non MADD	MADD	Non MADD	MADD
Delta	1 0	0 59	1 0	0 79	1 0	0 87
Log (delta)	1 0	1 0	0 99	1 0	0 98	1 0
Relative	1 0	0 31	1 0	0 62	1 0	0 76
Log (relative)	1 0	0 76	1 0	0 90	1 0	0 94
Integrated	1 0	0 60	1 0	0 79	1 0	0 87
Log (integrated)	1 0	1 0	0 99	1 0	0 98	1 0
	Percentage misclassifications					
Prevalence for MADD	0 041					
Loss factor L	1		10		100	
	Non MADD	MADD	Non-MADD	MADD	Non MADD	MADD
Delta	0*	12 5	0	0	0	0
Log (delta)	0	0	1 2	0	7 1	0
Relative	0	24 0	0	0	0	0
Log (relative)	0	12 5	1 2	0	1 2	0
Integrated	0	0	0	0	0	0
Log (integrated)	0	0	1 3	0	6 6	0

* Values in percentages

McArdle's Disease. Discrimination between McArdle patients and others turned out to be fairly easy in the standardized ischemic forearm test. All McArdle patients had a delta ammonia value of > 100 μmol/liter in combination with a delta lactate value of < 400 μmol/liter (Fig. 2).

DISCUSSION

Most authors in the literature have used an ischemic test procedure for forearm exercise.[1,6,8,10,13] Others prefer to work under nonischemic conditions.[2,12] Preliminary experiments indicated higher lactate and ammonia production in cases of ischemic exercise as compared to nonischemic exercise. For that reason, we developed a standardized ischemic forearm test.[17] This investigation shows that significantly greater amounts of lactate and ammonia are found in the standardized test than under nonstandardized conditions (Fig. 1). This holds true in controls and in patients with exertional myalgia.

From Fig. 2 it is evident that in order to discriminate between MAD-deficient patients and controls, a certain amount of lactate should be produced. A delta lactate value of 4.5 mmol/liter has been mentioned by Fishbein[2] in this respect. Taking into consideration the findings in Fig. 2, a delta lactate value of 2.5 mmol/liter is sufficient in our experience. Twenty-one percent of our patients had a lactate production of less than 2.5 mmol/liter with the unstandardized test. This percentage diminished to 12% under standardized conditions, thereby improving the value of this screening test. The test is indecisive in one of eight patients. Sometimes a repetition of the test proved to be helpful in these cases.

The appearance in time of lactate in venous blood samples after ischemic exercise is different from that of ammonia. In general, the ammonia level in venous blood peaked a little later than lactate. Also, between individual patients there were important differences in the time of appearance of maximal concentrations. Furthermore, it was observed that in some patients, lactate and ammonia remained relatively high during 7 minutes, whereas in others, there was a rapid decline to almost baseline values within that period. In clinical practice, it is therefore necessary to sample at various times after ischemic exercise.

A noteworthy finding was the unusually high ammonia production in three of our five McArdle patients (Fig. 2). A plausible explanation for this phenomenon has been put forward by Rumpf et al.[14] The muscular adenosine triphosphate (ATP) concentration in patients with McArdle's disease decreases rapidly upon ischemic exercise because of the enzymatic block in glyco(geno) lysis. The myokinase reaction generating 1 mol ATP and 1 mol AMP from 2 mol adenosine diphosphate (ADP) is enhanced by the decreased ATP/ADP ratio. In order to reach a further shift in the myokinase reaction in the direction of ATP synthesis, AMP is removed and converted into IMP by the enzyme MAD. ATP generated in this way can partially compensate for the lack of ATP production in the glycolysis. The increased flux through the myokinase reaction is to be considered as a compensation for the existing metabolic block. The increased deamination of AMP into IMP gives rise to a higher production

of ammonia in a muscle deficient in phosphorylase than in a normal muscle under the same conditions.

The interindividual variation in the shape of the ammonia and lactate curves could have implications for what turns out to be the most appropriate way of plotting lactate and ammonia values in order to obtain an optimal discrimination of MAD-deficient and non-MAD-deficient patients. For this reason, various ways of plotting the data have been used. Until now, delta values have been used by most authors.[3,6,12] Theoretically, it is to be expected that integrated values (i.e., a value approximating the total amount of metabolite produced during exercise) would result in a more sensitive discrimination of MAD-deficient and non-MAD-deficient patients. Discriminant analysis showed the results of these two ways of plotting (delta and integrated values) to be the best and to be equally good, as judged by the percentage of misclassifications and the values found for the posterior probabilities (Table 4). In consequence, there was no reason to initiate the use of integrated values. For the sake of both uniformity and simplicity, we propose to continue the use of (log)delta value in the interpretation of the results of the ischemic forearm test.

Sensitivity and specificity of the screening test depend on the discrimination line that is used in clinical practice (Fig. 2). None of our MAD-deficient patients had a delta ammonia value that exceeded the line $L = 1$. To be sure not to miss a deficiency in the future, it is proposed to use the line $L = 10$. This implies that the number of non-MAD-deficient patients suspected of having the deficiency will increase. Upon muscle biopsy, as the ultimate diagnostic procedure, these patients will prove not to be deficient for the enzyme. They have been false-positive in the screening test. It is quite possible that these false-positive tests refer to patients with the carrier status for the enzyme deficiency, which has been recently described by Fishbein et al.[4] Forty percent of the mean normal enzyme level was found in muscular tissue of MAD-deficient carriers, possibly giving rise to a lower ammonia production upon exercise. Type 1 fiber predominance could be an alternative explanation for relatively low ammonia/lactate ratios in the ischemic forearm test, as type 1 fibers are low in MAD activity.[2,4] Our data on the specificity and sensitivity of the ischemic forearm test are hard to compare with other studies in the literature, because the number of patients investigated in other studies is too small to give reliable results in this respect.[2,6,10,12]

Kelemen et al.[8] and DiMauro et al.[1] are uncertain about the usefulness of the forearm test as a screening test for MAD-deficiency. They describe a healthy volunteer in whom the ammonia level in venous return blood hardly increased with exercise.[1] We have found similar cases among our patients and controls. In these cases, however, we also found a low delta lactate value. This combination of results (low delta lactate plus low delta ammonia) means that the test is indecisive. We have observed that this can occur in healthy, well-motivated controls as well as in patients with exercise-related muscular complaints. In our opinion, muscular biopsy is indicated if repeated standardized ischemic exercise tests remain indecisive, although we realize that this does not prove that the patient has muscular pathology. The simultaneous measurement of lactate and ammonia thus contributes essentially to the value of this screening test. It allows the detection of MAD-deficiency, but moreover, it improves the screening for glyco(geno)lytic defects as well. For instance, McArdle

patients produce only little lactate but much NH_3. By the simultaneous measurement of both metabolites, they can be discriminated readily from patients that produce small amounts of both lactate and NH_3 during this test.

In summary, as shown in this study, the standardized ischemic exercise test with simultaneous measurement of lactate and ammonia proved to be an invaluable tool in the screening of patients with exercise-related muscular complaints.

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Chapter IV

ISCHAEMIC EXERCISE TEST IN MYOADENYLATE DEAMINASE DEFICIENCY AND McARDLE'S DISEASE: MEASUREMENT OF PLASMA ADENOSINE, INOSINE AND HYPOXANTHINE

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CLINICAL SCIENCE 70: 399-401, 1986

SUMMARY

1. Plasma adenosine, inosine and hypoxanthine concentrations were assayed in seven control subjects, five myoadenylate deaminase deficient (MADD)patients and six McArdle patients before and after ischaemic forearm exercise.

2. The plasma adenosine increase was very low in all test groups and there were no significant differences.

3. The MADD patients showed a significantly lower increase of plasma inosine and hypoxanthine after exercise as compared with the controls.

4. In the McArdle patients the increase in plasma inosine and hypoxanthine after exercise did not differ significantly from the values measured in the controls.

5. The ischaemic exercise test with measurement of plasma inosine and hypoxanthine might be of diagnostic value in MAD-deficiency, but not in McArdle's disease

INTRODUCTION

Recently the absence of plasma hypoxanthine elevation in response to forearm exercise was demonstrated in four myoadenylate deaminase deficient (MADD)-patients,[1] whereas an abnormal increase of hypoxanthine was reported in one McArdle patient.[2] In muscle biopsy material increased amounts of adenosine, inosine and hypoxanthine in the absence of the normal IMP accumulation after exercise was observed in one MADD patient. This increase in purine nucleosides and bases could, however, not account for the decrease found in the adenine nucleotide pools, and it was suggested that this might be the result of diffusion of the purine nucleosides and bases into the vasculature.[3]

This communication presents the results of an ischaemic exercise test with measurement of adenosine, inosine, and hypoxanthine in the venous return of the forearm in seven control subjects, five MADD patients and six McArdle patients.

SUBJECTS AND METHODS

The controls (four males, three females, age 22-52 years) were healthy volunteers without any complaint. The diagnosis in the MADD patients (five males, age 14-67 years) and the McArdle patients (two males, four females, age 8-37 years) was confirmed by histochemical and biochemical deficiency of myoadenylate deaminase and myophosphorylase, respectively. Additionally, a normal increase of plasma lactate and a subnormal increase of plasma NH_3 concentrations after ischaemic forearm exercise was found in each of the MADD patients, whereas a normal increase of plasma NH_3 and a subnormal increase of plasma lactate was found in each of the McArdle patients.

The control subjects, MADD patients and McArdle patients were subjected to a standardized ischaemic isometric forearm test as described earlier.[4] Blood samples were taken before and immediately after the test and at 3,5,7,9,11,15, and 20 minutes. By means of an indwelling catheter in the antecubital vein, 1.3 ml of blood was collected into 5 µl of heparin (500 i.u./ml) and 13 µl of 1 mmol/l deoxycoformycin (Warner-Lambert, Detroit, MI, U.S.A.), and immediately centrifuged at 9000 g for 1.5 minute. The plasma was collected and stored in liquid nitrogen without delay. The following extraction procedure was carried out at 4°C. After thawing, 400 µl of plasma was deproteinized with 100 µl of 2 mol/l $HClO_4$, and centrifuged at 6000 g for 3 minutes. Then 250 µl of the supernatant was neutralized with 45 µl of 3 mol/l $KHCO_3$ and again centrifuged at 6000 g for 3 minutes. The neutralized supernatant was analysed by high performance liquid chromatography using the method described by Hartwick et al.[5]

The data were evaluated statistically by the one-way analysis of variance according to Kruskal-Wallis. When the variance analysis yielded significant differences the Wilcoxon rank sum test for two groups was performed. The Dixon test was applied to show significant differences within a group. P values for two-tailed testing are given.

RESULTS

Table 1 presents the plasma concentration at rest, the maximal concentrations after exercise, the differences between these values, i.e., the increase in plasma adenosine (△Ado), inosine (△Ino), and hypoxanthine (△Hx) induced by muscular work, and the P values calculated according to Kruskal-Wallis. No significant P values were found with regard to all the plasma concentrations at rest, the maximal adenosine concentrations after exercise and △Ado, but significant P values were found with regard to the maximal inosine concentrations and △Ino, as well as the maximal hypoxanthine concentrations and △Hx.

The Wilcoxon test showed significant differences of △Ino and △Hx between MADD patients and controls ($P = 0.003$, $P = 0.003$). The ranges of the △Ino values

Table 1. *Effect of ischaemic forearm exercise on plasma purine nucleoside and base concentrations*
Plasma adenosine (Ado), inosine (Ino) and hypoxanthine (Hx): concentrations at rest, maximal concentrations after ischaemic forearm exercise, the differences between these values (all expressed in μmol/l) in controls, myoadenylate deaminase deficient (MADD) patients and McArdle patients, and P values calculated by the one-way analysis of variance according to Kruskal-Wallis.

	Age (years)	Sex	At rest			Maximal value after exercise					
			Ado	Ino	Hx	Ado	Ino	Hx	ΔAdo	ΔIno	ΔHx
Controls											
1	52	M	2.3	0.4	4.0	2.0	6.7	35.2	−0.3	6.3	31.2
2	29	M	0.2	0.5	1.7	0.6	17.8	38.5	0.4	17.3	36.8
3	22	F	0.0	0.6	1.6	0.0	11.7	27.2	0.0	11.1	25.6
4	23	F	0.0	0.6	0.6	0.0	15.6	25.6	0.0	15.0	25.0
5	33	F	0.0	0.6	0.6	0.0	9.2	24.3	0.0	8.6	23.7
6	34	M	0.2	1.1	1.3	1.5	4.4	32.9	1.3	3.3	31.6
7	32	M	0.4	0.9	0.9	0.9	8.7	40.5	0.5	7.8	39.6
Median	32		0.2	0.6	1.3	0.6	9.2	32.9	0.0	8.6	31.2
MADD patients											
1	47	M	0.9	0.0	0.7	6.1	0.4	9.4	5.2	0.4	8.7
2	24	M	0.9	0.4	1.1	1.7	0.9	8.7	0.8	0.5	7.6
3	67	M	0.7	0.2	0.9	3.9	0.9	8.3	3.2	0.7	7.4
4	14	M	1.1	0.2	0.4	2.6	1.3	6.3	1.5	1.1	5.9
5	29	M	0.4	0.9	1.1	0.4	0.9	10.4	0.0	0.0	9.3
Median	29		0.9	0.2	0.9	2.6	0.9	8.7	1.5	0.5	7.6
McArdle patients											
1	19	F	0.2	0.7	0.4	0.4	4.4	28.5	0.2	3.7	28.1
2	37	M	1.3	0.4	0.2	1.3	5.9	31.5	0.0	5.5	31.3
3	27	F	1.1	0.4	0.7	2.2	17.6	40.5	1.1	17.2	39.8
4	8	F	1.1	0.9	0.7	1.3	3.5	24.8	0.2	2.6	24.1
5	24	M	0.4	0.7	0.4	0.4	11.7	42.0	0.0	11.0	41.6
6	25	F	0.2	0.2	1.0	0.5	39.4	56.5	0.3	39.2	55.5
Median	24.5		0.75	0.55	0.55	0.9	8.8	36.0	0.2	8.25	35.55
P			0.12	0.16	0.08	0.09	0.006	0.005	0.10	0.006	0.004

were 0.4-1.3 μmol/l and 4.4-17 8 μmol/l and the ranges of the △Hx values were 6.3-10.4 μmol/l and 24.3-40.5 μmol/l in MADD patients and controls, respectively.

The △Ino and △Hx reached the highest values in McArdle patient no. 6. The Dixon test showed that this patient differed significantly from the other McArdle patients in △Ino ($P < 0.01$) but not in △Hx ($P < 0.1$). However, including or omitting the data from McArdle patient no. 6, no significant differences of △Ino and △Hx were found between McArdle patients and controls ($P > 0.1$, $P > 0.1$).

DISCUSSION

NH_3 is liberated in both the myoadenylate deaminase catalysed conversion of AMP to IMP and the adenosine deaminase catalysed conversion of adenosine to inosine. Consequently, inosine and hypoxanthine cannot be derived from AMP without NH_3 production. The subnormal plasma NH_3 increase shown earlier by Fishbein et al.[6] in MADD patients is, therefore, consistent with the subnormal plasma hypoxanthine elevation demonstrated by Patterson et al.,[1] and with our present findings. We were particularly interested in plasma adenosine concentrations because of the possibility that in myoadenylate deaminase deficiency AMP might be removed from the exercising muscle predominantly in the form of adenosine. For that reason we added deoxycoformycin to the blood samples in order to inhibit the conversion of adenosine to inosine by the adenosine deaminase reaction in whole blood. Even so in the MADD patients only a slight increase of plasma adenosine was found in response to ischaemic exercise (Table 1).

We should, however, take into account that by the determination of venous effluent concentrations we do not obtain a true picture of the production and release of metabolites by the working muscle. In that case one should also measure blood flow and arteriovenous differences. No attempt has been made to measure flow changes, but the comparable increase of lactate in controls and MADD patients after the exercise test suggests that the subnormal increase of inosine and hypoxanthine in MADD patients is probably not due to differences of the blood flow between controls and MADD patients. The absence of significantly increased adenosine concentrations in our MADD patients as compared with the controls might be explained by diminished ATP breakdown as reported by Sabina et al.[7] in three out of four MADD patients. Another possibility is the uptake of adenosine by endothelial cells.[8]

Our results confirm and extend the observations of Patterson et al.[1] and Brooke et al.[2] Besides a subnormal hypoxanthine response there was also a subnormal inosine response to ischaemic exercise in the MADD patients. A very high increase of inosine and hypoxanthine was measured in one McArdle patient, but a wide variation of these values was found in the McArdle patient group (Table 1). The measurement of plasma inosine and hypoxanthine before and after ischaemic exercise might be of value in the diagnosis of myoadenylate deaminase deficiency, but not in McArdle's disease.

ACKNOWLEDGEMENT

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Chapter V

AMP DEAMINASE DEFICIENCY: STUDY OF THE HUMAN SKELETAL MUSCLE PURINE METABOLISM DURING ISCHAEMIC ISOMETRIC EXERCISE

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CLINICAL SCIENCE (IN PRESS)

SUMMARY

1. Muscle biopsies were taken from 10 control subjects and 5 AMP deaminase (AMPD) deficient individuals before and after an ischaemic isometric exercise test and analysed for purine nucleotide, NAD^+, creatine phosphate (CP) and lactate content.

2. The decrease of ATP induced by the exercise test was significantly lower in the AMPD deficient patients than in the controls, but the decrease of creatine phosphate and the increase of lactate were not different. There were no significant differences in the exertional performance level between patients and controls and no evidence was obtained of an increased energy expenditure per unit of performance in AMPD deficiency.

3. The AMPD deficient individuals were equally capable to maintain a high adenylate energy charge (EC) as the control subjects, which indicates a normal regulation of the balance between ATP consumption and ATP regeneration.

4. ATP, ADP and total adenine nucleotide (TAN) but not AMP, were significantly elevated in the AMPD deficient patients as compared to the controls before as well as after the exercise test. This underlines the role of AMPD activity in the adenine nucleotide catabolism of skeletal muscle.

INTRODUCTION

Although AMP deaminase (AMPD, EC 3.5.4.6.) activity in skeletal muscle was discovered in the twenties and its absence was described for the first time as a coincidental finding in a case of periodic hypokalaemic paralysis in 1964,[1] it was not until 1978 that the deficiency state for this enzyme was thought to be associated with exercise intolerance.[2] Early fatiguability and exertional myalgia are considered to be the most prominent features of AMPD-deficiency.[3,4]

Together adenylosuccinate synthetase and -lyase and AMPD constitute the purine nucleotide cycle of which one important proposed function is the deamination of AMP (Fig. 1) in order to stabilize the adenylate energy charge during periods of ATP hydrolysis by pulling the adenylate kinase reaction towards ATP production.[5,6] The concept of the adenylate energy charge ($EC = \frac{ATP + \frac{1}{2} ADP}{ATP + ADP + AMP}$) has been introduced by Atkinson[6] and is thought to be a metabolic regulatory parameter especially in the regulation of the interaction between ATP regeneration and processes that consume ATP. In those situations where ATP utilization exceeds the resynthesis of ATP e.g. under ischaemic conditions, AMP deamination will occur.[7] The denominator of the EC is the size of the total adenine nucleotide pool (TAN = ATP + ADP + AMP) and can only decrease by the conversion of AMP. Two separate pathways are possible (Fig. 1): deamination to IMP by the activity of AMP deaminase or dephosphorylation to adenosine by the activity of 5'-nucleotidase.[8]

Sabina et al.[9] suggested that in the AMPD deficient muscle a lower capacity for energy production might be the cause of the clinical symptoms. They calculated that during a dynamic exercise test the median decrease in total phosphagen (i.e. ATP plus creatine phosphate) per unit of work in the AMPD-deficient patients was fivefold greater than in the control group. In their study however, they did not take into account the turnover of ATP associated with aerobic and anaerobic metabolism. Under non-ischaemic conditions such as during a dynamic exercise-test the aerobic and anaerobic ATP synthesis is partly derived from the breakdown of blood borne substrates and partly from the breakdown of muscle glycogen.

The present study was undertaken to look for a possible derangement in AMPD-deficiency of the adenine nucleotide metabolism which among others could be reflected by a change in the EC and, additionally, to see whether AMPD-deficient patients are facing a reduced energy economy. This paper reports on an ischaemic isometric exercise test and the levels of purine nucleotides, creatine phosphate and lactate assayed in muscle biopsies obtained before and after exercise from 5 AMPD-deficient individuals and 10 control subjects. Ischaemic conditions were adopted in order to minimize the ATP production associated with aerobic metabolism, to minimize the loss of lactate from the exercising muscle, and to prevent the resynthesis of creatine phosphate after muscle exertion.

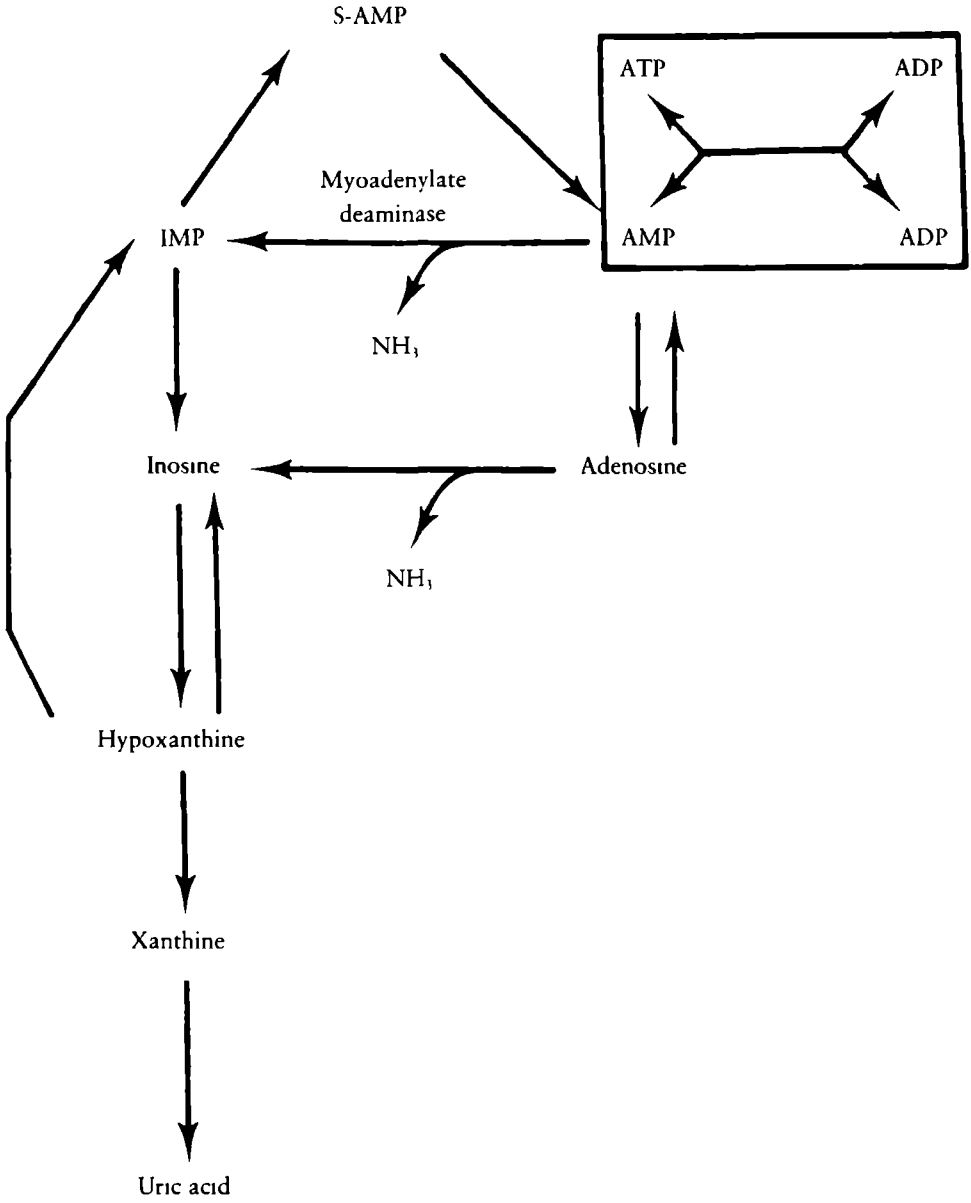

Figure 1. Metabolic pathways involved in the purine nucleotide cycle and the purine nucleotide catabolism.

MATERIALS AND METHODS

Subjects. 15 Subjects from whom informed consent had previously been obtained were involved in this study: 5 AMPD-deficient patients (4 males, age 24-48 years and 1 female, age 20 years) and 10 healthy volunteers (5 males, age 29-40 years and 5

females, age 23-33 years). The experimental protocol was approved by the committee of experimental research on human beings of the University of Nijmegen. After suspicion had been raised by the results of a standardized ischaemic forearm test[10] diagnosis of AMPD-deficiency was in all patients confirmed by histochemical and biochemical study of muscle biopsy material. All underwent this forearm test because of exercise intolerance. The residual AMPD-activity in these patients was less than 3% of the normal mean. Light microscopy and routine histochemical staining revealed no gross abnormalities except the absence of the AMPD. As a group the healthy volunteers were not especially well trained for this study but most of them regularly did some form of physical activity. Patient no. 3, an amateur motorcyclist, participated in an exercise program twice a week with muscle strength and endurance training.

Ischaemic isometric exercise test. The subjects were seated upright in a chair with the lower leg dependent and the knee flexed to 90°. The pelvis was secured by an adjustable belt. They were instructed to perform a voluntary isometric contraction with the knee-extensors at a force level of 50% maximal voluntary contraction force (MVC) until exhaustion, defined as a failure to hold the target force. This level has been chosen because it is known that peak accumulation of lactate by fatigue in isometric exercise occurs at 30 – 60% MVC.[11] The force generated by the knee-extensors was measured with a straingauge dynamometer secured just proximal to the malleoli and directly shown on a strip chart recorder in front of the subject. To quantitate the performance delivered, the impulse (I), that is the integral of force and time (Newton × min) of the quadriceps contraction was measured with a Kipp (Delft, The Netherlands) BC-1 integrator. The MVC was determined in three trials, interspaced by 1 minute, whereby the highest value which the subject could sustain for 1 second, was taken as the maximal voluntary contraction force. After a rest of 10 minutes local anaesthesia (1% Marcaine, Astra Pharmaceutics Rijswijk, The Netherlands) of the skin at the biopsy site, followed by incision was accomplished. A muscle sample was taken from the lateral part of the M. Quadriceps Femoris, with a modified Bergstrom needle. The biopsy material was removed from the needle with a forceps and immediately plunged into liquid nitrogen. The same procedure was repeated after exercise. The time that elapsed between the insertion of the needle at the end of exercise and freezing of the sample ranged from 15 to 25 sec. Ischaemia was brought on one minute before the start of the exercise by inflating a cuff arond the upper leg to 240 mm Hg which was deflated after the post-exercise biopsy had been taken.

Metabolite analysis. The frozen biopsy material was weighed, homogenized with ice cold 0.5 mol/l $HClO_4$ which was added in an amount such as to obtain a 10% W/V concentration, and centrifuged at 600 g for 5 minutes at 4°C. The supernatant was neutralized with 3 mol/l $KHCO_3$ and stored at −20°C.

ATP, ADP, AMP, IMP and NAD^+ in the neutralized supernatant were assayed by high performance liquid chromatography. A Spectra Physics (Santa Clara, California, USA) SP 8700 liquid chromatograph thermostated at 40°C equiped with a solvent programmer and a Rheodyne injection was used. The detector was a Spectra Physics

SP 8300 with a fixed wavelength of 254 nm. The signals were integrated electronically with a Spectra Physics SP 1400. A Chrompack (Middelburg, The Netherlands) Pellicular reverse phase precolumn (7.5 × 0.21 cm) was used and separation achieved on Chrompack μ-Bondapack C 18 column (30 × 0.46 cm) by a modification of the method described by Schweinsberg and Loo.[12] The column was eluated starting with 100% 0.1 mol/l K_2HPO_4/KH_2PO_4 buffer pH 6,25 at a flow rate of 0.6 ml/minute. At the time of injection of the sample, a linear gradient was started with methanol at a rate of increase of 1.5% methanol/minute until a concentration of 25.5% methanol was reached.

Creatine phosphate (CP) and lactate in the neutralized supernatant were measured by isotachophoresis as described earlier.[13] Instrumentation was a LKB (Stockholm, Sweden) Tachophor and a Kipp recorder.

The results of the measurements of purine nucleotides, CP and lactate were normalized to muscle NAD^+ content in order to decrease the variability due to differences in the composition of the muscle tissue obtained by muscle biopsy.[14] The energy (E) consumed in the ischaemic test procedure was calculated as the sum of the decrease in ATP and CP plus the increase in lactate multiplied with ½ because the production of 1 mole lactate from glycogen yields 1½ mole ATP.

Statistical analysis. The data were evaluated statistically by the two-way analysis of variance. *P*-values < 0.05 were considered significant.

RESULTS

The results are summarized in the Tables 1 – 3.

The two-way analysis of variance showed a significantly higher maximal voluntary contraction force (MVC) and impulse (I), but not endurance time (T), in the AMPD deficient and control males as compared to the AMPD deficient and control females (Table 1). These parameters were not significantly different in the AMPD deficient males and female as compared to the control males and females.
Skeletal muscle ATP, ADP and TAN, but not AMP, were significantly higher in the AMPD deficient males and female than in the control males and females before as well as after the exercise test (Table 2), while no differences were found between the AMPD deficient and control males as compared to the AMPD deficient and control females. The IMP content, however, was significantly lower in the AMPD deficient males and female than in the control males and females before but not after exercise, and no difference was found between the AMPD deficient and control males as compared to the AMPD deficient and control females. The EC was very slightly but significantly higher in the AMPD deficient and control males than in the AMPD deficient and control females before but not after exercise. There was, however, no significant difference between the AMPD deficient males and female as compared to the control males and females. Differences between the AMPD deficient males and

Table 1. Ischaemic isometric exercise test: age, sex, maximal voluntary contraction force (MVC), impulse (I) and endurance time (T) in the controls and the AMP deaminase deficient patients. The P values were calculated by the two-way analysis of variance (P is related to the control males and females versus the AMPD deficient males and female and P** to the control and AMPD deficient males versus the control and AMPD deficient females).*

Controls	*Age (yrs)*	*Sex*	*MVC (Newton)*	*I (Newton.min)*	*T (sec)*
1	*40*	*M*	*699*	*335*	*67*
2	*29*	*M*	*737*	*356*	*58*
3	*30*	*M*	*768*	*520*	*82*
4	*34*	*M*	*711*	*311*	*54*
5	*36*	*M*	*719*	*391*	*66*
Median	*34*		*719*	*356*	*66*
6	*23*	*F*	*493*	*204*	*50*
7	*32*	*F*	*512*	*238*	*62*
8	*23*	*F*	*401*	*164*	*53*
9	*23*	*F*	*386*	*164*	*58*
10	*27*	*F*	*413*	*248*	*74*
Median	*23*		*413*	*204*	*58*
Patients	*Age (yrs)*	*Sex*	*MVC (Newton)*	*I (Newton.min)*	*T (sec)*
1	*48*	*M*	*450*	*173*	*50*
2	*31*	*M*	*565*	*211*	*47*
3	*24*	*M*	*1016*	*460*	*56*
4	*36*	*M*	*607*	*341*	*63*
Median	*34*		*585*	*276*	*53*
5	*18*	*F*	*362*	*222*	*93*
*P**			*0.3573*	*0.2693*	*0.9120*
*P***			*0.0015*	*0.0076*	*0.5218*

female and the control males and females or between the deficient and control males and the AMPD deficient and control females were not detected with respect to skeletal muscle CP and lactate.

The ischaemic isometric exercise test caused a small but significantly lower decrease of ATP in the AMPD deficient males and female as compared to the control males and females (Table 3), but the decrease of CP and the increase of lactate were not different. The energy expenditure per unit of performance (E/I) calculated from the decrease of ATP and CP and the increase of lactate, was not found to be significantly higher in the AMPD deficient males and female than in the control males and females. No differences were detected between the AMPD deficient and control males as compared to the AMPD deficient and control females with any of these parameters.

DISCUSSION

In order to study the metabolism of skeletal muscle at work one can choose between a dynamic i.e. isotonic exercise test or an isometric exercise test, and also between ischaemic or non-ischaemic conditions. Independently of the test situation chosen, ATP is the ultimate energy source for contraction but it has to be continuously resynthesized. Various ways for the resynthesis of ATP are possible depending on the

Table 2 Skeletal muscle purine nucleotides (ATP, ADP, AMP, IMP), total adenine nucleotides (TAN) creatine phosphate (CP) and lactate concentrations (all expressed in nanomole/nanomole NAD^+), and the adenylate energy charge (EC) before and after exercise in controls and AMPD deficient patients The P values were calculated by the two-way analysis of variance (P is related to the control males and females versus the AMPD deficient males and female, and P** to the control and AMPD deficient males versus the control and AMPD deficient females)*

Contr	Sex	ATP	ADP	AMP	TAN	EC	IMP	CP	Lactate
1	M	14,2 – 8,9	2,00 – 1,90	0,01 – 0,10	16,21 – 10,9	0 94 – 0 90	0,30 – 5,61	50,1 – 8,6	11,4 – 87,3
2	M	12,3 – 9,9	1,43 – 1,47	0,04 – 0,12	13,77 – 11,49	0 95 – 0 93	0,22 – 1,94	58,2 – 12,7	6,2 – 57,3
3	M	12,3 – 11,6	1,58 – 1,95	0,09 – 0,02	13,97 – 13,57	0 94 – 0 93	0,12 – 0,46	57,9 – 10,7	5,6 – 40,3
4	M	12,3 – 11,9	1,45 – 1,76	0,08 – 0,07	13,83 – 13,73	0 94 – 0 93	0,18 0,81	48,2 – 10,3	4,1 – 39,5
5	M	12,8 – 12,2	1,81 1,91	0,01 – 0,03	14,62 – 14,14	0 94 – 0 93	0,25 – 0,75	53,4 – 29,9	6,6 – 34,0
Median		12,3 – 11,6	1,58 – 1,90	0,04 – 0,07	13,97 – 13,57	0 94 – 0 93	0,22 – 0,81	53,4 10,7	6,2 – 40,3
6	F	13,4 – 11,9	1,81 – 1,94	0,00 – 0,02	15,21 – 13,86	0 94 – 0 93	0,38 – 0,50	48,0 – 12,7	9,1 – 40,9
7	F	12,2 – 10,3	1,86 – 2,03	0,03 – 0,16	14,09 – 12,49	0 93 – 0 91	0,17 – 1,30	61,4 8,6	6,1 – 57,0
8	F	12,3 – 9,6	1,82 2,04	0,01 – 0,07	14,22 – 11,71	0 93 – 0 91	0,23 – 3,71	54,1 – 7,5	11,8 67,5
9	F	13,1 – 11,9	1,69 - 2,06	0,10 – 0,03	14,79 – 13,99	0 94 – 0 92	0,17 – 1,19	51,4 - 7,2	4,5 – 45,6
10	F	13,1 – 11,4	1,89 – 2,37	0,00 – 0,00	15,12 – 13,77	0 94 – 0 91	0,00 – 1,46	34,0 4 0	3,4 - 45,1
Median		13,1 – 11,4	1,82 – 2,06	0,01 – 0,03	14,79 – 13,77	0 94 – 0 92	0,17 – 1,30	51,4 – 7,5	6,1 – 45,6

Pat	Sex	ATP	ADP	AMP	TAN	EC	IMP	CP	Lactate
1	M	15,4 – 15,2	2,04 - 2,76	0,00 – 0,03	17,44 – 17,99	0 94 – 0 92	0,00 – 0,00	57,9 – 6,9	8,9 – 68,6
2	M	14,1 – 14,4	2,07 - 2,16	0,04 – 0,06	16,2 – 16,62	0 93 – 0 93	0,11 – 0,13	47,8 – 11,6	13,0 – 37,8
3	M	13,8 – 13,4	1,73 – 2,51	0,04 – 0,06	15,57 – 15,97	0 94 - 0 92	0,15 - 0,14	70,8 – 7,1	8,8 – 63,1
4	M	14,1 – 13,7	1,90 – 2,40	0,01 – 0,00	16,01 – 16,10	0 94 – 0 93	0,00 – 0,00	40,3 – 6,6	5,6 – 47,7
Median		14,1 – 14,1	1,97 – 2,46	0,02 – 0,05	16,1 – 16,36	0 94 – 0 93	0,06 – 0,065	52,9 – 7,0	8,8 – 55,8
5	F	14,1 – 14,6	2,16 – 2,07	0,09 – 0,04	16,35 – 16,71	0 93 – 0 94	0,09 – 0,11	57,2 – 26,1	9,7 – 42,9
P*		0 0022–0 0002	0 0267–0 0152	0 9937–0 3403	0 0022–0 0001	0 6394–0 2716	0 0307–0 0522	0 6487–0 9493	0 1806–0 9688
P**		0 9363–0 7532	0 0569–0 3615	0 9017–0 7472	0 7047–0 6110	0 0094–0 5876	0 8239–0 7993	0 6843–0 9085	0 8558–0 7284

type of exercise. During a dynamic non-ischaemic exercise test with increasing work loads such as the bicycle test used by Sabina et al.,[9] a considerable part of the ATP utilized is derived from oxidative phosphorylation especially in the beginning when using low loads. It is known that isometric contraction sustained at 20% or more of the MVC, impairs the muscle blood flow[15] thereby making substantial demand on anaerobic metabolism, which will be reflected in the lactate content of the muscle. Besides, under this condition predominantly fast-twitch-glycolytic type II B fibers will be used which have a higher AMPD-activity than the other fibre types.[16] We decided, therefore, to use an isometric test at 50% MVC to minimize the aerobic and to emphasize the anaerobic ATP production, and at the same time to activate especially the type II B fibers. Additionally we imposed ischaemic conditions arbitrarily starting the ischaemic period one minute before the exercise because it is known that after four minutes creatine phosphate already tends to decline.[17]

The ischaemic conditions imposed during our exercise-test allow a more precise estimation of the contribution of muscle glycogen breakdown to the total ATP turnover because no or at least a reduced amount of lactate is washed out of the exercised muscles. The lactate content of skeletal muscle in the AMPD-deficient patients was not significantly different from the lactate content in the controls (Table

Table 3 Delta values for ATP, creatine phosphate (CP) and lactate (all expressed in nanomole/nanomole NAD) after the ischaemic isometric exercise test, and the calculated energy consumed per unit of performance (EI, expressed in nanomole ATP/nanomole NAD⁺/Newton min) in controls and AMPD deficient patients The P values were calculated by the two-way analysis of variance (p is related to the control males and females versus the AMPD deficient males and female, and p* * to the control and AMPD deficient males versus the control and AMPD deficient females)*

Controls	*Sex*	*ΔATP*	*ΔCP*	*ΔLactate*	*EI*
1	*M*	*− 5,3*	*− 41,5*	*+ 75,9*	*4 80*
2	*M*	*− 2,4*	*− 45,5*	*+ 51,1*	*3 50*
3	*M*	*− 0,7*	*− 47,2*	*+ 34,7*	*1 92*
4	*M*	*− 0,4*	*− 37,9*	*+ 35,4*	*2 94*
5	*M*	*− 0,6*	*− 23,5*	*+ 27,4*	*1 67*
Median		*− 0,7*	*− 41,5*	*+ 35,4*	*2 94*
6	*F*	*− 1,5*	*− 35,3*	*+ 31,8*	*4 14*
7	*F*	*− 1,9*	*− 52,8*	*+ 50,9*	*5 51*
8	*F*	*− 2,7*	*− 46,6*	*+ 55,7*	*8 10*
9	*F*	*− 1,2*	*− 44,2*	*+ 41,1*	*6 53*
10	*F*	*− 1,7*	*− 30,0*	*+ 41,7*	*3 80*
Median		*− 1,7*	*− 44,2*	*+ 41,7*	*5 51*
Patients	*Sex*	*ΔATP*	*ΔCP*	*ΔLactate*	*EI*
1	*M*	*− 0,2*	*− 51,0*	*+ 59,7*	*8 14*
2	*M*	*+ 0,3*	*− 36,2*	*+ 24,8*	*3 46*
3	*M*	*− 0,4*	*− 63,7*	*+ 54,3*	*3 16*
4	*M*	*− 0,4*	*− 33,7*	*+ 42,1*	*2 85*
Median		*− 0,3*	*− 43,1*	*+ 48,2*	*3 31*
5	*F*	*+ 0,5*	*− 31,1*	*+ 33,2*	*3 62*
*P**		*0 0235*	*0 7337*	*0 7495*	*0 6570*
P * *		*0 7518*	*0 7928*	*0 6834*	*0 1130*

2 and 3), a finding which does not support the suggestion that AMPD-deficiency might interfere with the activation of phosphofructokinase by the ammonia produced in the AMPD reaction and, therefore, with the stimulation of the glycolysis during muscular exertion.[18] Our results also show that the AMPD-deficient individuals are capable of maintaining their EC at the same high level as the control subjects (Table 2), indicating a normal regulation of the balance of ATP consumption and ATP regeneration during ischaemic isometric exercise. This was also reflected by the decrease of skeletal muscle ATP after the exercise which was even lower in the AMPD deficient patients than in the controls (Table 3). A similar finding was reported by Sabina et al.[9] in 3 out of 4 AMPD deficient individuals using a dynamic exercise test.

As expected the ischaemic isometric test demonstrated a lower performance level in the females as compared to the males (Table 1). There was, however, no difference in performance level between the AMPD deficient patients and the controls in contrast to the finding of Sabina et al.[9] Previously, using a standardized ischaemic isometric handgrip test, we also found a normal performance in AMPD deficient patients but a subnormal one in myophosphorylase deficient (McArdle) patients.[19] This demonstrates that an ischaemic isometric exercise test is capable of discriminating between patients with different metabolic myopathies on the basis of differences between their level of performance.

If the decrease of skeletal muscle ATP and CP, and the increase of lactate was used to estimate the energy expenditure per unit of performance (E/I), then no significant difference was found between AMPD deficient individuals and control subjects (Table 3). The contribution of the residual aerobic metabolism to the ATP turnover under our ischaemic test conditions is unknown. One of the proposed functions of the purine nucleotide cycle is the production of fumarate which is an intermediate of the tricarboxylic cycle.[5] Dysfunction of the purine nucleotide cycle caused by AMPD deficiency might result in a reduced amount of fumarate and thus in a decreased residual aerobic metabolism in the patients. In that case the underestimation of E/I should be more pronounced in the controls than in the patients, i.e. the actual energy expenditure per unit of performance might then be lower in the patients than in the controls provided that the limitations imposed by our ischaemic test conditions still permit for sufficient residual metabolism.

The finding of higher skeletal muscle ATP, ADP and TAN concentrations in our patients as compared with the controls, before as well as after exercise (Table 2), is in agreement with the suggestion that AMPD activity plays an essential role in the catabolism of the skeletal muscle adenine nucleotides.[20] Previously, subnormal plasma hypoxanthine[20,21] and inosine[21] concentrations were found after forearm exercise in AMPD deficiency, but the adenosine level was not significantly different.[21] In vitro incubation studies reported by Sabina et al.[9] showed no evidence of adenosine production in muscle biopsies of controls in contrast to muscle biopsies from AMPD deficient patients and inhibition of the adenosine deaminase activity by 2-deoxycoformycine reduced the inosine- and hypoxanthine formation in AMPD deficient muscle biopsies but not in control muscle biopsies. These observations demonstrate that although the conversion of AMP to adenosine, inosine and hypoxanthine is possible in skeletal muscle, the catabolism of adenine nucleotides normally

involves conversion of AMP to IMP, inosine and hypoxanthine (Fig. 1). These observations also contradict another proposed function of the purine nucleotide cycle, that of preserving the purine nucleotide content of skeletal muscle through the accumulation of IMP during muscular exertion, thereby preventing the degradation of AMP to adenosine, inosine and hypoxanthine and the diffusion of these compounds out of the cell into the vasculature.[22]

The skeletal muscle IMP in our patients was significantly lower as compared to the controls before exercise. The difference after exercise was not significant ($P = 0.052$) due to the wide range of values obtained. Nevertheless, the tendency of only a small or even no increase of IMP in the patients was obvious (Table 2). Although the average increase in the control subjects was about 7-fold, it was by no means the 70 to 90-fold increase reported by Dudley and Terjung.[16] The high frequency stimulation used by them also caused a much larger decrease of TAN (50% versus 11%). The reason for this discrepancy could be the different type of exercise. An argument in favour of this supposition was provided by Edwards[15] who stated that depletion of energy substrates is not the limiting factor for duration of sustained voluntary isometric contractions.

The results of our study show that AMPD deficiency is not associated with an impaired energy economy during ischaemic isometric exercise. The possibility of a subnormal aerobic metabolism as a consequence of the failure to produce a sufficient amount of fumarate by the purine nucleotide cycle and, therefore, reduced ATP production during non-ischaemic isotonic exercise has not been investigated. Recently, however, Fishbein et al.[23] referred to the preferential association of AMPD with the sarcoplasmic reticulum and a possible role of the enzyme in the functioning of the calcium pump in the sarcoplasmic reticulum, the study of which might provide more insight in the pathophysiology of AMPD-deficiency.

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Chapter VI

MYOADENYLATE DEAMINASE DEFICIENCY: A CLINICAL, GENETIC AND BIOCHEMICAL STUDY IN NINE FAMILIES

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MUSCLE & NERVE (SUBMITTED)

SUMMARY

The clinical significance of myoadenylate deaminase (MAD) deficiency and its mode of inheritance are still questioned. Thirty-six relatives of 9 unrelated MAD-deficient patients were examined with the aid of a standardized ischemic forearm test. Eight new cases of MAD-deficiency were detected, 5 of which were confirmed histochemically and biochemically. Obligate heterozygotes showed a normal ammonia production and MAD-staining, but the mean activity of the enzyme was significantly less than in a group of controls. The results obtained from the family-study strongly suggest an autosomal recessive mode of inheritance. However, only 2 of the 8 newly found MAD-deficient individuals complained of exertional myalgia while the remaining 6 were without any symptoms or complaints. This finding casts doubt on the clinical significance of MAD-deficiency and the relationship of the deficiency state with exertional myalgia.

INTRODUCTION

In accordance with the proposed role of myoadenylate deaminase (MAD) in purine metabolism,[11] one would expect the complaints of deficient patients to be related to exercise. Exertional myalgia, fatigue and muscle pain, usually beginning in adult life, indeed are often mentioned [2 9] In some cases even exertional rhabdomyolysis has been described.[2 9,16]

However, complaints of exercise intolerance are rather common and unspecific, and in routine muscle biopsy series the deficiency of MAD is often detected (1-2% of muscle biopsies).[2] The association of MAD-deficiency with clinical complaints of exercise intolerance could therefore be coincidental. Furthermore, in about half the cases the deficiency is associated with other neuromuscular diseases.[2] It is not clear whether this is chance association or whether there is a MAD-deficiency secondary to the associated neuromuscular diseases. Consequently, several authors[13 14 16] question the clinical significance of the deficiency. The problem is further complicated by the fact that also symptomatic heterozygotes[1 6 7 9] are described, and even asymptomatic homozygotes without exercise-related complaints.[2 9] Although the frequency of occurrence of MAD-deficiency in muscle biopsy series is well known, there are no figures available about the prevalence in the general population. If MAD-deficiency is indeed only a harmless variant, then the occurrence in the population will be the same as in biopsy series. Kelemen et al.[9] found a frequency of 8 3% of MAD-deficiency in biopsies of patients with exertional myalgia, a frequency which was significantly higher than in the remaining biopsies in his series. This could point to a clinical significance of the deficiency and is not compatible with chance association. However, others could not confirm these findings [13]

The deficiency is probably inherited as an autosomal recessive trait.[9] Sibs of both sexes with the deficiency have been described,[9,15] and in one family an intermediate enzyme activity was reported in both parents of a deficient patient.[5] But also autosomal dominant inheritance[8 9] has been suggested. Even sex-linked recessive inheritance has been proposed,[7] but DiMauro et al.[1] have provided evidence that in the family reported, there was most probably an autosomal recessive inheritance. The aim of the present study is to obtain more information concerning the clinical significance of the deficiency (do newly diagnosed cases in the family study have exertional myalgia, do heterozygotes have complaints?) and its genetics. The need for diagnostic muscle biopsies could be reduced by the use of a sensitive screening test developed recently.[17] With this screening test the families of 9 patients with MAD-deficiency, who came to medical attention with exercise-related complaints without an associated neuromuscular disorder, were examined. New cases were detected, which were confirmed by histochemical and biochemical investigation of muscle biopsies. The data were compared with clinical symptoms and the outcome of a questionnaire on complaints.

MATERIALS AND METHODS

Patients and families.

We examined the relatives of 9 unrelated patients with MAD-deficiency and exertional myalgia but without associated neuromuscular disease (Table 1). All patients and their relatives gave their informed consent. The investigation had been

Table 1. Findings in probands

Proband of family no.	Age (yrs)	Sex	Complaints	Residual MAD-activity (% control mean)	Age (yrs) at time of onset of complaints
1	32	M	exertional myalgia	1.3%	25
2	20	F	exertional myalgia	1.3 %	3
3	38	M	exertional myalgia	< 1 %	37
4	50	M	exertional myalgia	1.6 %	42
5	17	M	exertional myalgia	< 1 %	8
6	15	F	exertional myalgia	15.1 %	14
7	26	M	exertional myalgia	< 1 %	13
8	33	M	exertional rhabdomyolysis	< 1 %	31
9	16	M	exertional myalgia	1.7 %	12

approved by the ethical committee of the St. Radboud Hospital. The residual MAD-activity in the muscle biopsy of the proband of family 6 was unusually high but because of the results of the ischemic exercise test and the absence, histochemically, of MAD-activity, she was included in the study. The proband of family 8 was seen because of severe rhabdomyolysis, his history gave evidence of exertional rhabdomyolysis. In all probands MAD-deficiency was histochemically and biochemically confirmed after suspicion had arisen in the ischemic exercise test. 36 Relatives (parents, spouses, sibs and children, Fig. 1) of our probands could be investigated with the aid of the standardized ischemic exercise test previously described;[17] only II 2 of family 7 could not be tested. Subsequently, from 6 out of 8 cases suspected of MAD-deficiency a needle biopsy was taken to confirm the diagnosis histochemically and biochemically. Additionally, 8 out of 20 obligate heterozygotes, (an autosomal recessive inheritance being assumed), underwent a needle biopsy to biochemically determine MAD-activity. All relatives had completed a questionnaire on exercise-related complaints, before the results of the exercise test were known. These questionnaires were rated blindly by one of the authors as evident, possible or no exercise-related complaints.

Biochemical studies.

MAD-activity was assayed in needle-biopsies of M. Quadriceps, essentially according to Leech and Newsholme,[10] by radiochemically following the conversion of [8−^{14}C] AMP (final concentration 2 mM) to [8−^{14}C] IMP. The value for normal mean MAD-activity was obtained by measuring enzyme activity in 8 muscle biopsies

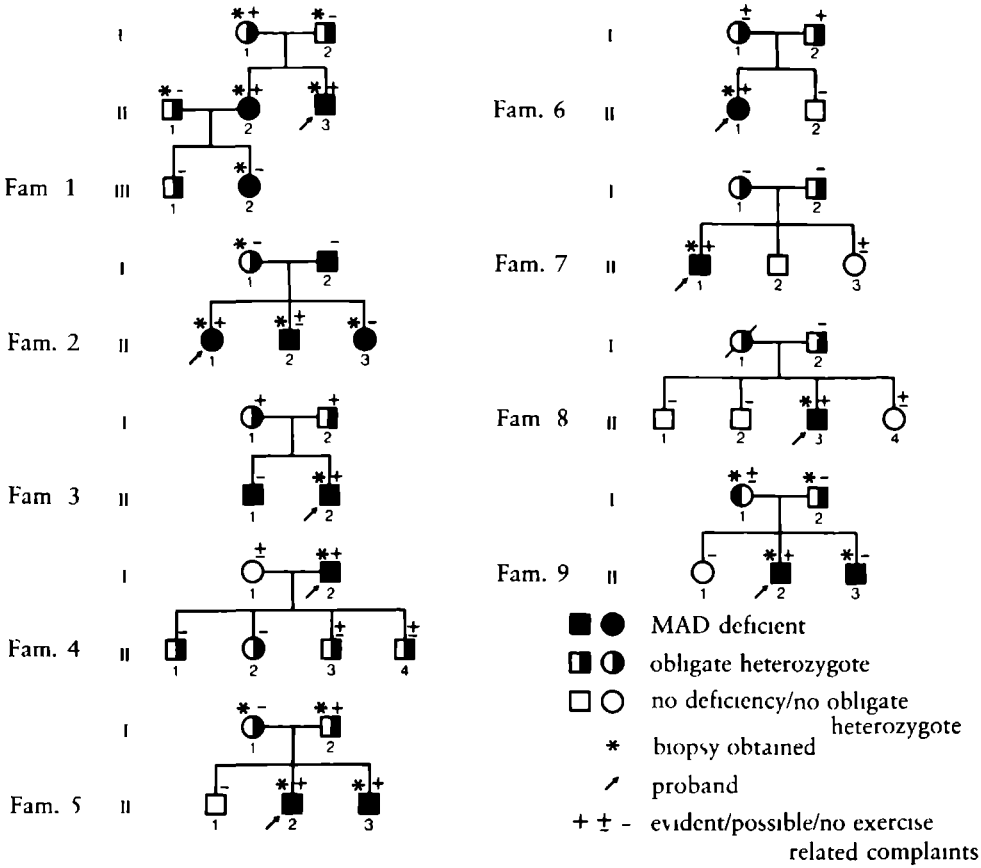

Fig 1 Pedigrees of 9 myoadenylate deaminase deficient patients

which showed no abnormalities on histochemical examination. Protein content was determined according to the method as described by Lowry et al.[12] The tissues were analyzed for histochemical MAD-activity with the technique described by Fishbein et al.[4]

RESULTS

New MAD-deficiency cases. By means of the ischemic exercise test 8 new cases were found to be suspect of MAD-deficiency (Fig. 2). This was subsequently confirmed histochemically and biochemically in most cases (Fig.1 and Table 2). In all cases studied, the results of the exercise test were consistent with these histochemical and biochemical data.

Genetic aspects. The new MAD-deficient cases were of both sexes (3 females and 5 males). In 7 out of 9 families the deficiency occurred only in sibs of the proband but

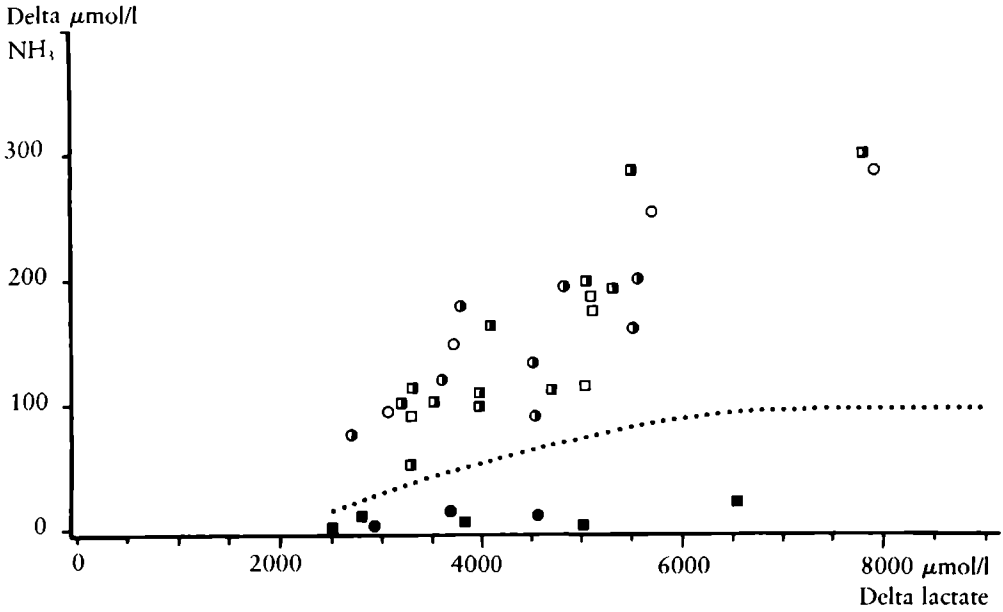

Fig.2. The results of the ischemic exercise test in 36 relatives of the 9 MAD-deficient index-cases. Symbols are given in figure 1, the line discriminates between MAD-deficiency and non-MAD-deficiency (Sinkeler et al.[7])

in two the deficiency state was found in successive generations (Family 1 and 2, Fig. 1). The deficiency occurred in 4 out of 11 sibs of probands with parents who were both thought to be heterozygotes (Family 1, III 1 and III 2, Family 2 and Family 4 were excluded). With the assumption that cases II 1 of Family 1 and I 1 of Family 2 are heterozygotes, the findings are compatible with an autosomal recessive trait. Enzyme activity in II 1 of Family 1, was more than 2 SD lower than the mean activity in controls, and in I 1 of Family 2, more than 1 SD.

Obligate heterozygotes. Because of the fact that the pedigrees shown can only be explained by an autosomal recessive inheritance, it is possible to designate 20 obligate heterozygotes. Obligate heterozygotes could not be detected with the exercise test since they showed a normal increase of ammonia after exercise (Fig. 2). Their muscle biopsies showed a normal MAD-staining. With the biochemical assay the mean MAD-activity in heterozygotes, 293 ± 57 nmol.min.$^{-1}$ per mg. protein (range 221-397, N = 8) was found to be significantly lower than the mean activity in the controls, 443 ± 95 nmol.min.$^{-1}$ per mg. protein (range 321-624, N = 8) ($P < 0.01$, Wilcoxon rank sum test). Individual heterozygotes could often not be designated due to the large standard deviation of the mean control value.

Complaints in new homozygotes and heterozygotes. Only 2 of the 8 new MAD-deficiency cases had complaints, as well as 5 of the 20 obligate heterozygotes (Table 2). None of the remaining 8 relatives, who are neither homozygotes nor obligate heterozygotes and among whom, possibly, some heterozygotes are present, had exercise-related complaints. Table 1 shows that in about half the cases of the probands (4/9) the MAD-deficiency did not present symptoms until adulthood, whereas it is evident from Table 2 that 5 out of the 8 newly found deficient individuals are still in their second decade.

Table 2. Findings in new cases of MAD-deficiency and obligate heterozygotes.

Fam.	*No.*	*Age (yrs)*	*Sex*	*Complaints*	*Age (yrs) at time of onset of complaints*	*MAD-stain*	*Residual MAD activity (% control mean)*
a. new deficiency cases:							
1,	II 2	35	F	+	12	absent	< 1 %
1,	III 2	11	F	−		absent	< 1 %
2,	I 2	52	M	−		n.d.	n.d.
2,	II 2	18	M	±	12	absent	< 1 %
2,	II 3	14	F	−		absent	< 1 %
3,	II 1	43	M	−		n.d.	n.d.
5,	II 3	9	M	+	6	absent	n.d.
9,	II 3	14	M	−		absent	< 1 %
b. obligate heterozygotes							
1,	I 1	58	F	+		present	56 %
1,	I 2	63	M	−		present	90 %
1,	II 1	38	M	−		present	50 %
1,	III 1	14	M	−		n.d.	n.d.
2,	I 1	46	F	−		present	73 %
3,	I 1	70	F	+		n.d.	n.d.
3,	I 2	70	M	+		n.d.	n.d.
4,	II 1	14	M	−		n.d.	n.d.
4,	II 2	19	F	−		n.d.	n.d.
4,	II 3	21	M	±		n.d.	n.d.
4,	II 4	22	M	±		n.d.	n.d.
5,	I 1	44	F	−		present	65 %
5,	I 2	48	M	+		present	56 %
6,	I 1	44	F	±		n.d.	n.d.
6,	I 2	41	M	+		n.d.	n.d.
7,	I 1	50	F	−		n.d.	n.d.
7,	I 2	56	M	−		n.d.	n.d.
8,	I 2	74	M	−		n.d.	n.d.
9,	I 1	42	F	±		present	66 %
9,	I 2	43	M	−		present	75 %

n.d. = not determined.
MAD-activity of controls: 443 ± 95 nmol.min^{-1} per mg. protein (N = 8, range 321-624).

DISCUSSION

As is shown by this family study (Table 3) only a minority of the newly diagnosed MAD-deficient family members and of the obligate heterozygotes have complaints of

Table 3. The relation of MAD-deficiency with exercise-related complaints.

Genetic state	Evident complaints	Possible complaints	No complaints
New homozygote	2	1	5
Obligate heterozygote	5	4	11
Not homozygote or obligate heterozygote	0	4	4

exertional myalgia. The complaints put forward had not been severe enough for them to call in medical attention. These complaints were not present in family members who were neither homozygotes nor obligate heterozygotes. From our findings it is evident that MAD-deficient individuals often do not have complaints. It should be mentioned, however, that it is not uncommon for MAD-deficient subjects to start getting complaints in adulthood.[3] Also in our probands (Table 1) this can be observed, but 5 out of 9 probands already had complaints in their first or second decade. From our 6 new deficiency cases without complaints, 4 are in their second decade (Table 2). It is possible that some of them will develop exercise-related complaints in the future, but it is unlikely that all will get complaints, taking into account that half of our probands complained of exertional myalgia already in their first or second decade.

So our family study does not demonstrate an evident relation between exercise-induced complaints and the deficiency state. Only part of the deficient individuals have complaints possibly related to the deficiency. The same considerations do hold, in our opinion, for the heterozygotes.

MAD-deficiency is reported to be an autosomal recessive defect. The results of our family study are compatible with this mode of inheritance. In two families (Fam. 1 and 2), however, there are deficiency cases in successive generations (Fig. 1). Assuming that family 1, II 1 and family 2, I 1, are heterozygotes, these family trees can also be explained with an autosomal recessive mode of inheritance. This assumption is supported by the finding of a reduced MAD-activity in the biopsies of these relatives (Table 2). The finding of the two families with pseudo-dominant inheritance points to a high frequency of heterozygotes in the population; Fishbein et al.[2] calculated on the basis of the Hardy-Weinberg law and a frequency of MAD-deficiency in the biopsy population of 1-2%, a frequency of heterozygotes of 18-24%. If MAD-deficiency should be indeed only a clinically insignificant biochemical finding, then the frequency of heterozygotes in the general population could be in the same order of magnitude. The finding in our family study of two pseudo-dominant inheriting families is in accordance with a high frequency of heterozygotes in the general population. The assumption of an autosomal recessive mode of inheritance is further supported by our finding of a significantly lowered mean MAD-activity in obligate heterozygotes compared with controls, as was observed earlier in one family.[5]

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Chapter VII

MYOADENYLATE DEAMINASE DEFICIENCY: CAUSE OF EXERTIONAL MYALGIA OR INCIDENTAL FINDING?

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MUSCLE & NERVE (SUBMITTED)

SUMMARY

The clinical significance of myoadenylate deaminase (MAD) deficiency is still questioned. Most authors favour the idea of MAD-deficiency being a cause of exertional myalgia. Retrospectively, the occurrence of MAD-deficiency in 519 routine muscle biopsies was determined and compared in a prospective study with the prevalence of MAD-deficiency among 217 patients with exercise-related complaints in order to see whether MAD-deficiency occurs more frequently in an exertional myalgia group, which wasn't the case. A group of healthy volunteers was studied for comparison. Recently we reported on 8 newly found MAD-deficient individuals among 36 relatives of 9 MAD-deficient patients. Of these 8 MAD-deficient subjects, however, only 2 had exercise-related complaints.

The findings cast doubt on the association between MAD-deficiency and exertional myalgia.

INTRODUCTION

Myoadenylate deaminase (MAD) deficiency was for the first time causally related to a metabolic myopathy in 1978 by Fishbein et al.[4] In large series of consecutive muscle biopsies, executed for a broad spectrum of neuromuscular disorders, the frequency of occurrence of the deficiency ranged from 1 – 2.9% which made it the most common known enzyme deficiency of skeletal muscle.[5,10 11,13,17] Soon after the first report it became clear that there were no distinctive clinical features among the patients although the majority complained of exercise-related symptoms such as fatigue, cramps or myalgias, commonly denoted as 'exertional myalgia'.

Even though it was not possible to distinguish the disorder from other pathologic entities by means of the uncharacteristic and subjective complaints of the patients, in ascertaining the clinical significance of the deficiency the functional and metabolic abnormalities in MAD-deficiency found by Sabina et al.[15] seemed promising. The authors concluded from their experiments that patients with MAD-deficiency tire more quickly and perform less work than normal controls or other patients with exertional myalgia do. As previously described[20] and in contrast to these findings, we could not find any difference between MAD-deficient patients and controls. Neither could we obtain evidence,[18] as judged by the purine nucleotide, creatine phosphate and lactate content of muscle biopsies before and after exercise, of an increased energy expenditure per unit of performance in MAD-deficiency as suggested by Sabina et al.[15] In other words, the assumption of myoadenylate deaminase deficiency being the cause of exertional myalgia or exercise intolerance is still not substantiated.

Contributing to further confusion is the fact that absence of MAD-activity has also been described in patients with a heterogenous group of unrelated conditions, such as hypokalaemic periodic paralysis,[2] amyotrophic lateral sclerosis,[11] Kugelberg-Welander syndrome,[17] progressive systemic sclerosis,[7] facial and limb girdle myopathy[14] and so on. Especially Shumate,[16 17] but also Hayes et al.[8] and Heffner[9] questioned the pathologic significance of MAD-deficiency and suggested that the deficiency was an incidental finding or produced symptoms only in concert with other, as yet unidentified, abnormalities or circumstances. However, in a retrospective survey of 302 muscle biopsies, Kelemen et al.[11] found 36 biopsies which were performed on index cases with myalgia. All these biopsies were investigated with the aid of a histochemical MAD-stain.[6] MAD-deficiency was significantly more common in this exertional myalgia cohort than it was in a group of patients who underwent a muscle biopsy for other clinical problems On the basis of these retrospective data, the authors admitted a relationship between MAD-deficiency and myalgia.

The discrepancies between the positive findings of Kelemen et al. concerning the clinical relevance of MAD-deficiency based on a rather small group of patients with exertional myalgia and our results as mentioned before, prompted us to study prospectively the frequency of occurrence of the deficiency-state in a large group of patients with exercise-related complaints and in a group of healthy volunteers, in an attempt to answer the question whether or not MAD-deficiency is associated with exercise-related complaints.

SUBJECTS AND METHODS

Study 1. In the years 1980-1986, 519 patients, over 10 years of age who had been referred with a broad spectrum of neuromuscular complaints, underwent a muscle biopsy. Approximately 30% of the biopsies executed at our institution is from patients below the age of 11 and with the majority in the first half of that decade. Since these youngsters do not reliably indicate exertional myalgia it seemed more appropriate to exclude them from the study. In family studies (a.o. malignant hyperthermia) only the proband was included. From study 1 were also excluded patients with exercise-related complaints, because we intended to look for the prevalence in 2 patient-cohorts: those with exercise-related complaints (study 2) and those without (study 1). The muscle tissues were analyzed for histochemical MAD-activity with the technique as described by Fishbein et al.[6] In the 519 specimens, the intensity of staining on gross and microscopic examination was the criterion used for diagnosis. If MAD-deficiency was detected histochemically and sufficient biopsy material was available, then MAD-activity was assayed biochemically. We did not take into account the possibility of secondary deficiencies as suggested by Fishbein et al.[3] because residual MAD-activity was practically absent in most cases.

Study 2. In the years 1983-1986, 217 patients performed a standardized ischemic forearm test, as previously described,[19,20] because of their exercise-related symptoms. In these cases where the test suggested a MAD-deficiency or was equivocal in this respect, a biopsy followed in order to substantiate the deficiency histo- and biochemically. Biochemically MAD-activity was measured essentially according to the method described by Leech and Newsholme.[12]

Study 3. The 78 healthy volunteers consisted of 45 males (mean age 32 yrs, range 19-55) and 33 females (mean age 30 yrs, range 22-55), mainly hospital personnel and medical students. They also performed the standardized ischemic forearm test.

RESULTS

Study 1. In the retrospective survey of 519 biopsy specimens, 11 lacked enzyme-activity histochemically i.e., 2.1%. The biochemical enzyme-activity was determined in 9 cases (Table 1) which all had a residual MAD-activity of less than 6.9% of the control mean value (105 nmol/mg protein per hour, range 30-311, N = 20). In the remaining 2 patients there was not enough material to determine the enzyme-activity biochemically (Table 1).

Study 2. In the prospective study 217 patients performed the screening test of which 10, i.e., 4.6%, produced an insufficient amount of ammonia suggesting a MAD-deficiency which was subsequently histochemically and biochemically confirmed in all cases (Table 2).

Table 1 Eleven retrospectively found MAD-deficient patients: some characteristics.

Patient nr.	*Age (yrs)*	*Sex*	*Clinical or biopsy finding*	*Residual MAD-activity (% control mean)*
1	*69*	*M*	*polyneuropathy*	*0.9*
2	*34*	*M*	*acid maltase deficiency*	*2.0*
3	*37*	*M*	*dystrophia myotonica*	*6.2*
4	*59*	*M*	*polyneuropathy*	*1.1*
5	*55*	*M*	*polyneuropathy*	*< 0.5*
6	*22*	*M*	*CK-elevation*	*0.8*
7	*53*	*F*	*polyneuropathy*	*6.9*
8	*13*	*M*	*polyneuropathy*	*< 0.5*
9	*61*	*M*	*polyneuropathy*	*< 0.5*
10	*48*	*F*	*polyneuropathy*	*N.D.*
11	*69*	*F*	*polymyositis*	*N.D.*

N.D. = Not Determined

Table 2. Ten prospectively found MAD-deficient patients with exertional myalgia.

Patient nr.	Age (yrs)	Sex	Residual MAD-activity (% control mean)
1	32	M	1.3
2	20	F	1.3
3	38	M	< 1
4	50	M	1.6
5	17	M	< 1
6	15	F	15.7
7	26	M	< 1
8	33	M	< 1
9	16	M	1.7
10	53	M	< 1

When comparing the prevalence figures in the retrospective study 1 and the prospective study 2, which are 2.1% and 4.6% respectively, using a Chi^2-test, we find that these figures are not significantly different ($P = 0.11$).

Study 3. In 2 out of 78 healthy volunteers, i.e. 2.6%, the forearm test was strongly suggestive of MAD-deficiency. These two male volunteers were aged 25 and 29 years. They did not have any complaints and both tolerated (prolonged) physical exercise well. Also total impulse during the exercise test was normal.

DISCUSSION

Up to now the clinical significance of myoadenylate deaminase deficiency is still questioned. If the deficiency is not responsible for a definite clinical disease syndrome, one would expect the prevalence of this genetic curiosity to be the same in the normal population as in muscle biopsy series.[1] However, the determination of the prevalence

in the general population is clearly not well possible and for that reason did we and others approximated this figure by studying a large series of muscle biopsies executed for all kinds of neuromuscular complaints but excluding exercise-related ones.
The 2.1% occurrence of histochemical absence of MAD in our series is in accordance with the prevalence of MAD-deficiency reported by several authors.[10,11,13,17] MAD-deficiency occurred in the exertional myalgia group somewhat more frequently (4.6%) than in the routine muscle biopsies (2.1%). The difference, however, does not reach statistical significance and so could be due to chance. Our supposition that MAD-deficiency occurs in the general population as frequent as in muscle biopsy series is encouraged by the finding of 2 deficient subjects out of 78 healthy volunteers i.e. 2.6%. It is obvious, however, that this latter cohort is not a representative sample of the general population.
Anyhow, when inspecting the above mentioned findings it becomes doubtful whether MAD-deficiency is associated with exertional myalgia. This conclusion is unlike that of Kelemen et al.[11] The cause of the discrepancy lies mainly in the prevalence figures obtained in the exertional myalgia group (3 MAD-deficient patients out of 36 in his material versus 10 out of 217 in ours).
There are several explanations:

1. it is possible that in our prospective exertional myalgia study some MAD-deficient cases remained undetected due to a low sensitivity of the screening-test. However, as previously described,[20] this is highly unlikely.
2. the threshold for patients to be selected for muscle biopsy is higher than for exercise testing. So, possibly, in our prospective exertional myalgia group more patients have been included with a.o. complaints of psychogenic origin, than in the retrospective muscle biopsy group of Kelemen et al.,[11] which lowers the frequency of occurrence of MAD-deficiency in the prospective study.
3. MAD-deficiency is rather often associated with a moderate CK-elevation.[4] It is not impossible that in the selection of patients for muscle biopsy in Kelemen's study this elevation has played a role and thus his exertional myalgia group might be enriched with MAD-deficiency. In our selection for exercise testing this CK-elevation did not play a role.
4. children do not often complain explicitly of exertional myalgia. So the inclusion of children in the reference muscle biopsy group of Kelemen et al. might be a cause of distortion of the prevalence figures.

Summarizing the data, there is for the moment no statistically significant evidence that MAD-deficiency is seen more often in exertional myalgia, the supposed clinical syndrome of MAD-deficiency. If there is a relation between MAD-deficiency and exertional myalgia, then this concerns only a minority of the MAD-deficient cases as is evident from the discovery of only 2 deficient symptomatic relatives out of 8 deficient relatives in a family survey[21]. Therefore in many cases MAD-deficiency seems to be a harmless genetic variant. The question of the clinical relevance of MAD-deficiency still awaits a better understanding of the physiologic and metabolic consequences of the deficiency.

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Chapter VIII

CONCLUDING REMARKS

In this study we have tried, as outlined in Chapter I, to answer some questions that have arisen with regard to a recently described new enzyme deficiency of skeletal muscle: myoadenylate deaminase deficiency.[1]
The lack of a strongly suggestive set of clinical signs or symptoms and the supposed high frequency of occurrence made it necessary to refine the screening method originally suggested by Fishbein.[1] Because it was early recognized that the main source of the ammonia produced during exercise was the reaction catalyzed by myoadenylate deaminase (MAD),[2] the plasma determination of ammonia (together with lactate, as an independent estimate of the intensity of muscular work), constitutes the pillar of the screening method.

Chapters II and III deal with the questions which force level and frequency of contractions should result in blood concentrations of lactate and ammonia that discriminate between patients and controls, and whether standardization of the ischaemic exercise test would improve screening in MAD-deficiency.

Up to now a major problem in interpreting the results of ischaemic exercise tests has been the assessment whether the subject performed sufficient work. Help in determining if sufficient work has been performed during the ischaemic test comes from studies, documenting a linear relationship between increases in ammonia and lactate during exercise in normal subjects.[1,3,5] In case there is a subnormal elevation of lactate as well as of ammonia, it is now possible, with the concurrent measurement of lactate and ammonia and because of this linear relationship, to denote an inadequate work-performance. By measuring only lactate, as McArdle[6] did, it was not possible to differentiate between a glyco(geno)lytic defect or just an insufficient work-performance. Thus the concurrent measurement of lactate and ammonia levels during ischaemic exercise performance should be of considerable assistance in evaluating patients suspected to have a metabolic disorder of muscle function as. (1) it can help establish whether the work performed was an adequate challenge for the glyco(geno)lytic pathway or the purine nucleotide cycle (PNC), and (2) it has potential to identify patients with metabolic disorders that may present the symptoms of exertional myalgia (e.g. MAD-deficiency, McArdle's disease). Our study shows that standardization improves screening in MAD-deficiency, but we do not pretend to have arrived at the optimal challenge for the PNC of which AMP deamination is one step. Although most arguments are in favour of maximal enzyme activity during ischaemic exercise,[7-9] there is one proposed function of the PNC that does not come into play under ischaemic conditions. This is the production of fumarate, an intermediate of the oxydative citric acid cycle.[10] Therefore, it is imaginable that the consequences of MAD-deficiency are shown most prominently when muscles are exercising under more or less aerobic conditions. In this case a non-ischaemic test device would deserve attention. Nevertheless, the standardized ischaemic forearm test has been shown to be a very sensitive screening method in MAD-deficiency.

The supposed pathophysiological consequences of MAD-deficiency might be associated with a disruption of the PNC.[11] Several mechanisms have been suggested to

explain the role of the PNC in skeletal muscle:[2 10]

1. the increase of the flux through the PNC could serve to maintain the energy-charge (EC) during muscular contraction;
2. the ammonia production might stimulate phosphofructokinase and enhance the rate of glycolysis;
3. the PNC could serve to replenish fumarate, a citric acid cycle intermediate, which might be important during an increased demand for ATP;
4. the accumulation of IMP during exercise might preserve the skeletal muscle purine nucleotide pool by preventing the degradation of AMP to adenosine, inosine and hypoxanthine.

The measurements of several metabolites in plasma (chapter IV) and muscle biopsies (chapter V) from MAD-deficient patients throw some light on these proposed mechanisms.

ad 1. disruption of the PNC caused by MAD-deficiency does not result in an inability to maintain the EC at a normal level during ischaemic isometric exercise.

ad 2. the reduced production of ammonia in MAD-deficiency does not prevent the normal stimulation of glyco(geno)lysis as shown by the skeletal muscle lactate concentrations after exercise.

ad 3. the effect of a reduced fumarate generation on the ATP production of MAD-deficient muscle can probably not be studied under ischaemic, i.e. anaerobic conditions such as in our test because, as mentioned before, fumarate can only serve as a fuel when oxygen is available.

ad 4. the hypothesis that IMP accumulation during muscular contraction preserves the purine nucleotide pool is not supported by the finding in MAD-deficient patients of an elevated skeletal muscle total adenine nucleotide content and a subnormal increase of plasma inosine and hypoxanthine after exercise.

The results of the studies described in chapter IV and chapter V have not provided any evidence of how the disruption of the purine nucleotide cycle might cause the symptoms of exertional myalgia. In view of the very high skeletal muscle MAD-activity, however, it is quite possible that MAD has a certain as yet unrecognized function which may or may not be associated with the PNC. Perhaps future studies on MAD-deficiency will reveal this function.

Finally, chapter VI and chapter VII deal with the question whether MAD-deficiency manifests itself with exertional myalgia or not. In solving this problem two approaches have been used First, the relatives of known MAD-deficient patients were screened for MAD-deficiency after they had completed a questionnaire on exercise-related complaints. In this way 8 new MAD-deficient subjects were discovered of whom only 2 complained explicitly of exertional myalgia. This means that probably only a minority of MAD-deficient individuals in the population will complain of exertional myalgia. Additionally, it became evident that MAD-deficiency shows an autosomal recessive mode of inheritance. The findings pointed to a high frequency of heterozygotes in the general population.

In the second approach, the frequency of occurrence of MAD-deficiency in a

series of muscle biopsies executed because of not exercise-related complaints, was compared with the prevalence found in a patient cohort with exercise-related complaints. Although the frequency of occurrence of MAD-deficiency is higher in the exertional myalgia group than in the muscle biopsy series, the difference does not reach the level of significance. This is in disagreement with the findings of Kelemen et al.[12] However, as evidenced in chapter VII there are many questions unresolved concerning the findings of Kelemen et al. We are convinced that for the moment there is no evidence for a relation between exertional myalgia and MAD-deficiency. It is possible that MAD-deficiency does not produce symptoms unless in concert with other, as yet unknown abnormalities or circumstances. Such an abnormality could be, for instance, an altered fiber-type distribution in MAD-deficiency. The finding of the highest activity of MAD in type II B fibers[13] and type II fiber predominance in muscle cramp and exertional myalgia[14] both point to this possibility. In the study described in chapter V all controls (N = 10) and symptomatic patients (N = 5) were biopsied. Analysis of variance, however, revealed no significant difference ($P > 0.05$) between controls and patients in relation to fiber-type distribution.

Until now we have not commented on the frequent association of MAD-deficiency with other neuromuscular disorders and on the subsequent question whether this association is simply coincidental, or occurs as a consequence of the neuromuscular disorder. In this context a primary form of MAD-deficiency means a coincidental association of MAD-deficiency with other neuromuscular diseases whereas a secondary form of MAD-deficiency refers to the deficiency-state being the consequence of another disorder. Fishbein[15] states that there is definitely a secondary form of MAD-deficiency, in which all of the muscle enzymes are lowered by pathologic damage, but adenylate deaminase is lowered out of proportion to the others. This statement is based on the following facts: (1) an associated muscle disease is much more likely in patients with a high residual MAD-activity, or, in other words, the lower your residual enzyme activity, the more likely you are to have pristine deficiency with no other neuromuscular pathology; and (2) the residual MAD-activity in secondary cases shows significant, although incomplete, removal by antibody, whereas, rabbit antisera to the purified human skeletal muscle isozyme do not react with the residual adenylate deaminase activity of primary deficient muscle biopsies.[16]
When the first strategy is applied to our material i.e. when the cases of MAD-deficiency are sorted according to their residual MAD-activity, we do find associated neuromuscular disorders in patients with low residual MAD-activity (Table 1). This points to chance association of MAD-deficiency with other neuromuscular diseases and probably to a high prevalence of MAD-deficiency not only in muscle biopsy series but also in the general population. So our results do not show the relation between associated neuromuscular pathology and the high residual enzyme activity as reported by Fishbein.[13]

When considering all previous findings, it cannot be ruled out that MAD-deficiency is only a harmless, frequently occurring genetic curiosity without exercise-related symptoms. Nor do we consider an altered purine metabolism, as exists in MAD-deficiency judging by the plasma-hypoxanthine concentration and purine-nucleotide content of muscle after exercise, to explain the complaints. A better

understanding of the metabolic and physiological consequences of the deficiency will probably bring the clinical relevance of the deficiency in some cases to evidence.

Table 1. MAD-deficient subjects sorted according to their residual adenylate deaminase activity.

Patient number	*Clinical finding or Histopathology*	*Residual MAD activity (% control mean)*
2.	*exertional myalgia*	< 0.5
4.	*exertional myalgia*	< 0.5
6.	*exertional myalgia*	< 0.5
7.	*exertional myalgia*	< 0.5
11.	*exertional myalgia*	< 0.5
16.	*exertional myalgia*	< 0.5
18.	*neuropathy*	< 0.5
21.	*neuropathy*	< 0.5
22.	*neuropathy*	< 0.5
28.	*neuropathy*	< 0.5
31.	*none*	< 0.5
33	*none*	< 0.5
34.	*none*	< 0.5
36.	*none*	< 0.5
30.	*none*	0.6
10.	*exertional myalgia*	0.8
37.	*none*	0.9
13.	*neuropathy*	0.9
25.	*muscular dystrophy*	0.9
17.	*neuropathy*	1.1
26.	*muscular dystrophy*	1.2
3.	*exertional myalgia*	1.3
1.	*exertional myalgia*	1.3
5.	*exertional myalgia*	1.7
12.	*exertional myalgia*	1.8
14.	*acid maltase deficiency*	2.0
24.	*muscular dystrophy*	3.8
23.	*muscular dystrophy*	4.0
15.	*myotonic dystrophy*	6.2
20.	*neuropathy*	6.9
9.	*exertional myalgia*	15.7

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APPENDIX

Several case-studies are presented with the intent to illustrate (1) the supposed clinical picture of MAD-deficiency (case 1 and 2), (2) the difficulty in some cases to define the limits of exertional myalgia and to impute muscle fatigue to MAD-deficiency (case 3) and (3) the association of MAD-deficiency with other neuromuscular disorders (case 4 and 5).

All the patients in this study are listed in Table 1.

Table 1
MAD-deficiency with the clinical feature of exertional myalgia

Patient number	Sex	Age at time of onset of complaints (yrs)	CK	Residual MAD activity (% control mean)
1.	M	25	39	1 3
2.	F	12	18	0.5
3.	F	3	34	1.3
4.	M	37	132	0.5
5	M	42	44	1.7
6.	M	16	47	0.5
7.	M	8	161	0.5
8.	M	5	101	N.D
9.	F	14	31	15.7
10.	M	13	134	0.8
11.	M	31	58	0.5
12.	M	12	109	1.8

MAD-deficiency with an associated neuromuscular disorder and exertional myalgia

Patient number	Sex	Age at time of diagnosis (yrs)	CK	Histopathology	Residual MAD activity (% control mean)
13	M	68	37	neuropathy	0.9
14.	M	34	178	acid maltase defic.	2 0
15.	M	36	540	myotonic dystrophy	6.2
16.	M	53	94	focal motor neuron loss	0.5
17.	M	58	67	neuropathy	1 1
18.	M	57	84	neuropathy	0 5
19.	F	35	496	myotonic dystrophy	N.D.
20	F	52	39	neuropathy	6.9
21.	M	12	164	neuropathy	0.5
22.	M	60	42	neuropathy	0.5

CK value normal, less than 100 U/l.
N.D. = not determined

MAD-deficiency with an associated neuromuscular disorder without known exertional myalgia

Patient number	Sex	Age at time of diagnosis (yrs)	CK	Histopathology	Residual MAD activity (% control mean)
23.	M	0	68	cong. dystrophy	4.0
24.	M	7	6110	dystrophy	3.8
25.	M	3	155	dystrophy	0.9
26.	M	2	3000	dystrophy	1.2
27.	M	0	76	cong. dystrophy	N D
28.	M	4	96	neuropathy	0.5
29	F	69	943	myositis	N.D.

Patient number	Sex	Age at time of diagnosis (yrs)	CK	Residual MAD activity (% control mean)
30	M	2	85	0 6
31	F	11	46	0 5
32	M	51	29	N D
33	M	17	279	0 5
34	F	14	52	0 5
35	M	43	127	N D
36	M	13	52	0 5
37	M	34	112	0 9
38	M	47	N D	N D
39	M	42	N D	N D
40	M	25	104	N D healthy volunteer
41	M	29	86	N D healthy volunteer

CASE REPORTS

1. *Patient no 1*

A 32-year-old man was the second child of non-consanguineous parents. He was referred in 1982 with complaints of generalized muscle aches after exertion since 3 years. Before that time he could run a marathon without great difficulties but nowadays, he stated to 'hit the wall' about half-way. He noticed a slight progression of the complaint and also that chewing caused aching of the jaw muscles. His history gave no evidence of a second wind phenomenon or pigmenturia. The only and elder sister of the patient was more severely disabled by having complaints already when climbing a flight of stairs. In the family-study (chapter VI) she also appeared to be MAD-deficient.

Physical examination nor routine chemical blood- and urine analysis of this male revealed any abnormality. A right quadriceps muscle biopsy specimen showed no abnormalities except for the absence of histochemical MAD-activity.

Conclusion: familial coincidence of exertional myalgia with MAD-deficiency.

2. *Patient no 10*

This 26-year-old man came to medical attention when aged 21 years because of a progressive exercise-intolerance which he suffered from since childhood. As an amateur motor-crosser he experienced soreness and stiffness in both his forearms during races, especially when the weather was cold. They were relieved in half an hour of rest. His history gave no evidence of pigmenturia; there was no family-history of any muscle disease.

Clinical examination revealed no abnormalities, blood examination repeatedly showed mildly elevated plasma creatine kinase levels (134-168 U/l; normal values up to 100 U/l). An EMG showed the presence of some small polyphasic potentials of short duration. In a right quadriceps muscle biopsy specimen the absence of MAD-staining was demonstrated but showed otherwise no abnormalities.

Conclusion: lifelong exertional myalgia.

3. *Patient no. 5*

A 50-year-old man was hospitalized at age 46 for severe and permanent muscle-aches in the left lower-leg. He experienced an exercise-induced progression of this complaint.

On examination, muscle bulk was normal and there was no weakness at rest. The lumbar spine functioned without abnormal findings. Blood examination revealed no abnormalities, an EMG showed no evidence of nerve entrapment. X-ray studies showed a first degree listhesis of L5 with the lumbar sub-arachnoideal space having a diameter just within normal limits. Except for absent adenylate deaminase staining, a biopsy of the left gastrocnemius muscle was normal.

Conclusion: (pseudo) radicular syndrome or MAD-deficiency?

4. *Patient no. 18*

This 59-year-old male was referred in 1983 with suspicion of a neuropathy. He complained of progressive and painful paraesthesia of both feet since 1982. He also had, however, a 20-year history of easy fatiguability and leg pains induced by exercise. The patient ascribed these complaints to a myocardial infarction in 1964.

Physical examination was normal except for decreased sensibility in both lower-legs. EMG and nerve conduction studies were consistent with an axonal polyneuropathy. A left sural nerve biopsy specimen showed a decrease in myelinated fibers whereas a left soleus muscle biopsy showed type-grouping and sporadic nuclear aggregation with mild endomysial connective tissue proliferation. The MAD-staining was absent.

Conclusion: primary MAD-deficiency together with a polyneuropathy.

5. *Patient no. 29*

A 70-year-old woman had suffered from a stiff back and muscle weakness of the arms for 8 months. Besides, she noticed an aching discomfort in her shoulders.

Physical examination showed no abnormalities except for a mild generalized muscle weakness. Serum-transaminase, LDH and CK were elevated and suggested a polymyositis which was confirmed in a quadriceps muscle biopsy specimen. MAD-staining was absent.

Conclusion: polymyositis with absent MAD-staining and without exertional myalgia.

SUMMARY

This thesis deals with the investigation into an enzyme deficiency in muscle which has only recently been described but has, nevertheless, a high prevalence: myoadenylate deaminase deficiency. This enzyme deficiency is often associated with complaints of aching muscles, muscle spasm, and abnormal fatiguability due to physical exertion, commonly denoted as exertional myalgia. The results of this study are laid down in six papers discussing the following three aspects of the disease:
- the screening for the deficiency, avoiding the necessity of a biopsy,
- the biochemical changes during exercise that might account for the complaints,
- the clinical significance of the deficiency.

The aim of *chapter I* is to outline the framework from which the questions, formulated at the end of the chapter, originate. It contains a description of the conflicting opinions found in literature as to the deficiency's clinical significance. The conflict is based on the absence of a specific pattern of complaints and on the fact that the enzyme deficiency is often an incidental, secondary finding in other neuromuscular disorders. The enzyme's place as a part of the purine nucleotide cycle is discussed, as well as the relevance of this cycle for the energy economy within the muscle and its consequences for the development of the screening methodology. We decided to use the ischaemic forearm test in combination with measurement of lactate and ammonia levels before and after exertion. The main source of ammonia during muscular exertion is the ammonia freed in the conversion of AMP into IMP by the enzyme which is the focus of this study: myoadenylate deaminase. Lactate is measured because it serves as an independent variable for the muscle exertion performed. Next, the various views on the mode of inheritance are dealt with. Finally, the objectives of the study are worded in six questions, the answers to which are given in the following chapters.

Chapter II describes the search (by means of an ischaemic forearm test) for the combination of strength and frequency resulting in the highest possible lactate and ammonia levels. A frequency of 30 isometric contractions per minute with a voluntary contraction force of 80% of the maximal strength has turned out to be the combination producing the best results.

Chapter III is about the comparison of specificity and sensitivity of this standardized test and a not-standardized one, with regard to the detectibility of the enzyme deficiency. Furthermore the different ways of plotting the results of the standardized test are compared, in order to be able to discriminate between deficient and not deficient individuals. Surprisingly, deficient individuals have proved to be able to perform the same amount of 'work' in these exercise tests as healthy volunteers.

In *chapter IV* the measurements are stated of a number of purine nucleotide degradation products in venous blood of healthy volunteers and patients. These measurements have been carried out before as well as after the individuals performed the standardized ischaemic forearm test. It is apparent that patients with enzyme

deficiency produce considerably fewer degradation products of AMP (inosine and hypoxanthine). From this it is concluded that the alternative route of removing AMP from the deficient muscle by conversion into adenosine is not used to a large extent in this test.

Chapter V deals with the changes in the tissue concentrations of energy-rich compounds before and after an isometric contraction of the quadriceps muscle. Measurements in enzyme-deficient patients and healthy volunteers show that there are no differences between both groups as to (1) the work-performance and (2) the amount of energy used per unit of work. This implies that by means of an ischaemic isometric test it cannot be demonstrated that the enzyme-deficient patient is at disadvantage compared to a healthy person as far as his energy economy is concerned. Yet in the deficient group, both before and after exercise, purine nucleotide concentrations, with the exception of AMP, are higher than those in the volunteers.

Neither *chapter III*, nor *chapters IV* and *V* provide evidence of some disorder in the purine nucleotide cycle which might explain the clinical symptomatology usually associated with the deficiency.

Chapter VI enters into the clinical and genetic aspects of the enzyme-deficiency by means of a family survey. Thirty-six relatives of 9 probands are examined for a possible deficiency with the aid of the standardized ischaemic exercise test.

Eight new deficient relatives are discovered, only 2 of whom, however, have exercise-related complaints. This finding casts doubt on the enzyme-deficiency's clinical significance regarding exercise-related complaints. The data obtained from the survey are in accordance with an autosomal recessive inheritance.

In *chapter VII* the question of the deficiency's clinical significance is approached by way of determining the enzyme-deficiency's prevalence in three different patient populations and comparing the results. Prevalence was established in: (1) a routine series of muscle biopsies, carried out to diagnose a variety of neuromuscular disorders with the exception of biopsies done on account of exertional myalgia, (2) a patient cohort just because of their exercise-related complaints, as this is the presumed clinical syndrome in the enzyme deficiency. This group of patients was examined by means of the standardized ischaemic exercise test. In cases where the tests suggested a MAD-deficiency a muscle biopsy was performed in order to corroborate this, (3) a group of healthy volunteers, also by means of the screening test mentioned before.

Comparison of the different prevalences showed that the deficiency does not have a statistically significant higher prevalence in the group of patients with exercise-related complaints, and that it also occurs in a group without complaints.

Finally, in *chapter VII* we comment on the functions of the purine nucleotide cycle as they are described in literature but do not seem to be confirmed by the findings of our own investigations. Moreover, the chapter goes into the association of the enzyme-deficiency with other neuromuscular diseases, which we consider to be based mainly on coincidence and it is not secondary to the primary disease.

We have come to the conclusion that myoadenylate deaminase deficiency is a frequently occurring, harmless genetic variant, which does not necessarily induce exercise-related complaints.

SAMENVATTING

Dit proefschrift omvat het onderzoek naar een eerst onlangs (1978) beschreven maar veelvuldig voorkomende enzym-deficientie in skeletspieren: de myoadenylaat deaminase deficientie. Deze enzym-deficientie wordt in verband gebracht met 'exertional myalgia', klachten over spierpijn, spierkramp en abnormaal snelle vermoeibaarheid ten gevolge van inspanning. De zes artikelen waarin de resultaten van deze studie hun neerslag vinden, hebben betrekking op een drietal aspecten van de aandoening, te weten:

- de screening op de aanwezigheid van de deficientie zonder dat een biopt noodzakelijk is,
- de biochemische veranderingen tijdens inspanning die de klachten zouden kunnen verklaren,
- de klinische betekenis van de deficientie.

Hoofdstuk I beoogt het kader aan te geven van waaruit de aan het eind van het hoofdstuk geformuleerde vragen, zijn ontstaan. Het beschrijft de tegengestelde meningen welke in de literatuur bestaan ten aanzien van de klinische betekenis van de deficientie doordat er ten eerste, geen specifiek klachtenpatroon aanwezig is, en ten tweede, het ontbreken van het enzym herhaaldelijk een bijkomende bevinding is bij neuromusculaire aandoeningen. Besproken worden de plaats van het enzym in de purine nucleotide cyclus en het belang van deze cyclus voor de energie-huishouding van de spier met de daaruit voortvloeiende consequenties voor de te ontwikkelen screenings-methodiek. Gekozen wordt voor een gestandaardiseerde ischaemische handknijptest met lactaat en ammoniakbepalingen vóór en na inspanning. De belangrijkste bron van ammoniak tijdens spierinspanning is het ammoniak dat gevormd wordt bij de omzetting van AMP naar IMP door het enzym dat centraal staat in dit onderzoek: het myoadenylaat deaminase (MAD). Lactaat wordt gemeten omdat het dient als een onafhankelijke resultante van de verrichte spierinspanning. Hierna komen een aantal onduidelijkheden ter sprake betreffende de wijze van overerven. Tot slot worden de doelstellingen van het onderzoek beschreven welke zijn vervat in een zestal vragen waarvan de beantwoording plaatsvindt in de successievelijke hoofdstukken.

In *hoofdstuk II* wordt het zoeken, met behulp van een ischaemische handknijptest, beschreven naar die combinatie van kracht en frequentie waarmee de hoogste lactaat- en ammoniakwaarden verkregen kunnen worden. Een frequentie van 30 isometrische contracties per minuut met een krachtsniveau van 80% van de maximale kracht blijkt de combinatie welke leidt tot de hoogste waarden.

Hoofdstuk III is gewijd aan de vergelijking van de specificiteit en sensitiviteit van deze gestandaardiseerde en niet-gestandaardiseerde testopstelling met betrekking tot de mogelijkheid om de enzym-deficientie vast te stellen. Tevens worden de verschillende wijzen waarop de uitkomsten van de gestandaardiseerde test kunnen worden uitgezet vergeleken, teneinde zo goed mogelijk te kunnen discrimineren tussen defi-

cienten en niet-deficienten. Verrassenderwijze blijken deficienten tijdens deze inspanningstest evenveel 'arbeid' te kunnen verrichten als gezonde proefpersonen.

In *hoofdstuk IV* worden de bepalingen vermeld van een aantal purine nucleotide degradatieproducten in veneus bloed bij gezonde proefpersonen en patienten. Deze metingen worden verricht zowel vóór als na het uitvoeren van de gestandaardiseerde ischaemische handknijptest. Het blijkt dat patienten welke enzym-deficient zijn, beduidend minder afbraakproducten van AMP (inosine en hypoxanthine) produceren. Hieruit wordt geconcludeerd dat de alternatieve mogelijkheid om de AMP concentratie te verlagen via de omzetting in adenosine in de deficiente spier bij deze testopstelling niet in belangrijke mate gebruikt wordt.

In *hoofdstuk V* worden de veranderingen behandeld in de spierweefsel-concentraties van AMP, ADP, ATP, CP en lactaat vóór en na een isometrische contractie van de musculus quadriceps. De metingen bij enzym-deficienten en gezonde proefpersonen maken duidelijk dat er geen verschillen bestaan tussen de beide groepen wat betreft (1) de geleverde inspanning en (2) de hoeveelheid verbruikte energie per eenheid van inspanning. Dit wil dus zeggen dat met deze ischaemische, isometrische test-opstelling niet aangetoond kan worden dat de enzym-deficiente patient wat betreft zijn energie-huishouding in het nadeel is ten opzichte van gezonde proefpersonen. Evenwel zijn in de deficiente groep de concentraties purine-nucleotiden, met uitzondering van AMP, zowel vóór als na de inspanning hoger dan bij de proefpersonen.
Zowel in *hoofdstuk III* als in *hoofdstuk IV* en *V* worden geen aanwijzingen gevonden voor een ontregeling van de energie huishouding die de klinische symptomatologie, welke gewoonlijk aan de deficientie wordt gekoppeld, zou kunnen verklaren.

In *hoofdstuk VI* wordt ingegaan op de klinische- en genetische aspecten van de enzym-deficientie aan de hand van familie-onderzoek. 36 Verwanten van 9 probandi worden met behulp van de gestandaardiseerde ischaemische inspannings-test onderzocht op de mogelijkheid van vóórkomen van de deficientie.
Er worden 8 nieuwe deficienten ontdekt waarvan er echter maar 2 inspanningsgebonden klachten hebben. Deze bevinding doet twijfel ontstaan aan de klinische betekenis van de enzym-deficientie voor wat betreft de aan inspanning gerelateerde klachten. De uit de studie verkregen gegevens zijn in overeenstemming met een autosomaal-recessieve erfgang.

In h*oofdstuk VII* wordt de vraag naar de klinische betekenis van de deficientie benaderd door in een drietal populaties de prevalentie voor deze enzym-deficientie te bepalen en met elkaar te vergelijken. De prevalenties zijn nagegaan: (1) in een routine serie spierbiopten welke zijn verricht voor de diagnostiek van een aantal neuromusculaire aandoeningen, met uitzondering van die biopten welke verricht werden alleen vanwege 'exertional myalgia', (2) in een patienten-cohort met juist dergelijke aan inspanning gerelateerde klachten omdat dit het veronderstelde klachtensyndroom is bij de enzym-deficientie. Deze patientengroep werd onderzocht met de gestandaardi-

scerde ischaemische inspanningstest. Indien deze test wees op een MAD-deficientie dan werd ter bevestiging een spierbiopsie verricht en (3) in een groep gezonde proefpersonen eveneens met behulp van de eerder vernoemde screenings-test.

Vergelijking van de verschillende prevalenties leert ons dat de deficientie niet statistisch significant méér frequent aanwezig is in de groep met aan inspanning gebonden klachten en dat de deficientie ook in een groep zonder klachten voorkomt.

In *hoofdstuk VIII* tenslotte worden een aantal kanttekeningen geplaatst bij de in de literatuur veronderstelde functies van de purine nucleotide cyclus welke door onze onderzoeksbevindingen niet bevestigd worden. Het gaat tevens in op het samengaan van de enzym-deficientie met andere neuromusculaire aandoeningen, hetgeen ons inziens veelal berust op toeval en niet secundair is aan de primaire aandoening.

Wij komen tot de conclusie dat de myoadenylaat deaminase deficientie een veelvuldig voorkomende, onschuldige genetische variant is welke niet noodzakelijkerwijze leidt tot aan inspanning gebonden klachten.

PARTS OF THIS STUDY HAVE BEEN OR WILL BE PUBLISHED:

Sietze P. T. Sinkeler. Hein A. M. Daanen, Ron A. Wevers, T. Lian Oei, Ed M. G. Joosten and Rob A. Binkhorst.
The relation between blood lactate and ammonia in ischemic handgrip exercise.
MUSCLE & NERVE 8:523-527, 1985.

Sietze P.T. Sinkeler, Ron A. Wevers, Ed M. G. Joosten, Rob A. Binkhorst, T. Lian Oei, Martin A. van 't Hof and Anton F. de Haan.
Improvement of screening in exertional myalgia with a standardized ischemic forearm test.
MUSCLE & NERVE 9:731-737, 1986.

S. P. T. Sinkeler, E. M. G. Joosten, R. A. Wevers, R. A. Binkhorst, F. T. Oerlemans, C. A. van Bennekom, M. M. Coerwinkel and T. L. Oei.
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CLINICAL SCIENCE 70:399-401, 1986.

S. P. T. Sinkeler, R. A. Binkhorst, E. M. G. Joosten, R. A. Wevers, M. M. Coerwinkel and T. L. Oei.
AMP deaminase deficiency: study of the human skeletal muscle purine metabolism during ischaemic isometric exercise.
CLINICAL SCIENCE (IN PRESS)

Sietze P. T. Sinkeler, Ed M. G. Joosten, Ron A. Wevers, T. Lian Oei, Alga E. M. Jacobs, Jaques H. Veerkamp and Ben C. J. Hamel.
Myoadenylate deaminase deficiency: a clinical, genetic and biochemical study in nine families.
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Sietze P. T. Sinkeler, Ed M. G. Joosten, T. Lian Oei and Ron A. Wevers.
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De ischaemische onderarmtest in de neuromusculaire diagnostiek.
TIJDSCHRIFT NEDERLANDSE VERENIGING VOOR KLINISCHE CHEMIE 11:50-54, 1986.

CONTRIBUTIONS TO MEETINGS:

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Abstracts published in: *CLINICAL PHYSIOLOGY*
S. P. T. Sinkeler, R. A. Wevers, R. A. Binkhorst, E. M. G. Joosten, T. L. Oei.
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Skeletal muscle adenosine, inosine and hypoxanthine release following ischemic forearm exercise in myoadenylate deaminase deficiency and McArdle's disease.
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Myoadenylate deaminase deficiency and McArdle's disease: plasma adenosine, inosine and hypoxanthine after ischemic forearm exercise.
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CURRICULUM VITAE.

De schrijver van dit proefschrift werd op 29 juni 1949 te Nijmegen geboren. Aldaar doorliep hij de gemeentelijke H.B.S.-B en volgde aan de Universiteit van Nijmegen de opleiding tot arts. Het arts-examen werd op 25 juni 1976 behaald.
De opleiding tot zenuwarts, van 1976 – 1981, vond eveneens plaats te Nijmegen: in het Sint Radboud Ziekenhuis op de afdelingen neurologie (Prof. Dr. J. J. G. Prick) en psychiatrie (Prof. Dr. S. J. Nijdam) en in het Canisius Ziekenhuis op de afdeling neurochirurgie (Prof. Dr. H. A. D. Walder).
Op 1 oktober 1981 vond inschrijving plaats in het register voor zenuw- en zielsziekten waarna hij enkele maanden werkzaam was als consulent neuroloog en psychiater in het psychiatrisch ziekenhuis Vrederust in Halsteren.
De aantekening 'Klinische Neurofysiologie' (opleiders: Prof. Dr. S. L. H. Notermans en Drs. P. J. H. Bernsen) werd op 1 april 1983 behaald.
Sinds 1 april 1983 is hij als psychiater verbonden aan de Nederlands Hervormde Diaconessen Inrichting te Meppel.

STELLINGEN

behorende bij het proefschrift

MYOADENYLATE DEAMINASE DEFICIENCY

I

De afwezigheid van klachten bij personen die myoadenylaat deaminase deficient blijken te zijn, noopt ertoe de betekenis van de purine nucleotide cyclus voor de skeletspier tijdens inspanning aan een herbeschouwing te onderwerpen.

II

Bij myoadenylaat deaminase deficientie is het onwaarschijnlijk dat tijdens inspanning een abnormale toename van de bloeddoorstroming in de spieren optreedt.

III

De betekenis van het tijdens inspanning middels oppervlakte electroden geregistreerde myogram voor het inzicht in de pathofysiologie van 'exertional myalgia', wordt onvoldoende gewaardeerd.

IV

Bij het onderzoek naar de oorzaak van inspanningsgebonden klachten dient betrokken te worden in hoeverre de klachten een gevolg kunnen zijn van een slechte lichamelijk conditie respectievelijk trainingstoestand.

V

Resectie van de eerste rib als therapie voor een 'thoracic outlet' syndroom wordt veelal ten onrechte toegepast.

VI

De dementie geassocieerd met de ziekte van Parkinson wordt waarschijnlijk veroorzaakt door een stoornis in meerdere neurotransmitter systemen.

VII
Het bezigen van de uitdrukking 'vlucht in de waanzin' ter verklaring van sommige psychosen veronderstelt ten onrechte wilsvrijheid.

VIII
De verscheidenheid aan psychopathologie van verschillende en gewaardeerde schrijvers doet vermoeden dat de waardering wel samenhangt met hun psychopathologie maar niet gebonden is aan één specifieke vorm.

IX
Het primaat van het lichaam in de fraaie versregels uit Slauerhoff's gedicht 'Het boegbeeld· de ziel':
Dit is mijn lot: gebeeldhouwd voor den boeg,
Den scheepsromp achter mij te moeten volgen;
brengt de dialectische wederkerigheid van lichaam en ziel op een niet gebruikelijke wijze uit balans.

X
'It is better to travel hopefully than to arrive'.

S. P. T. Sinkeler

Langenboom, 23 januari 1987